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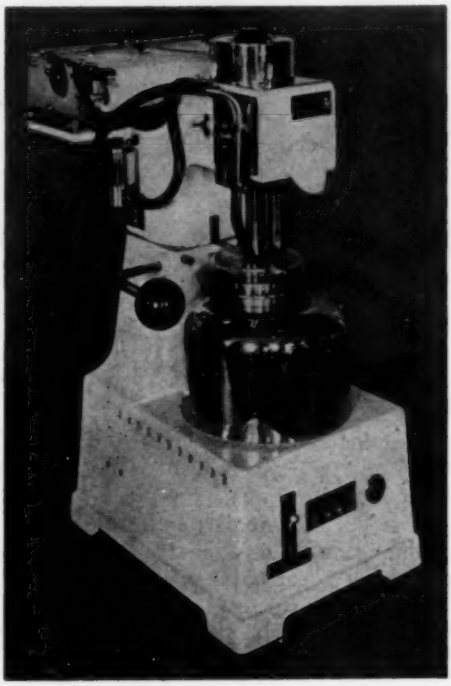

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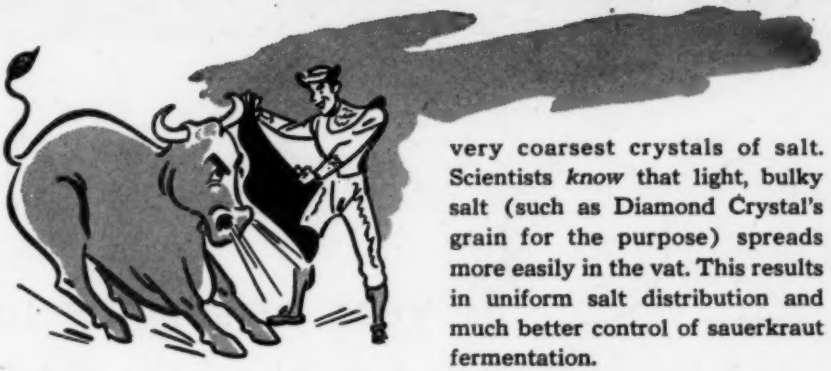

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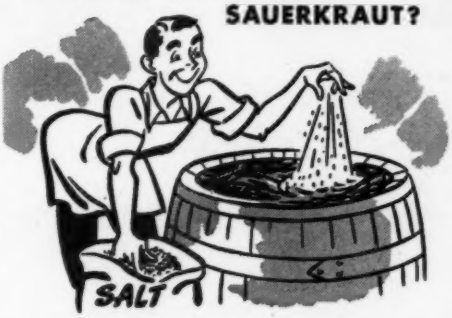

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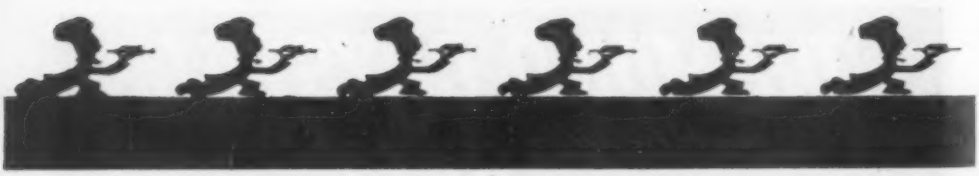

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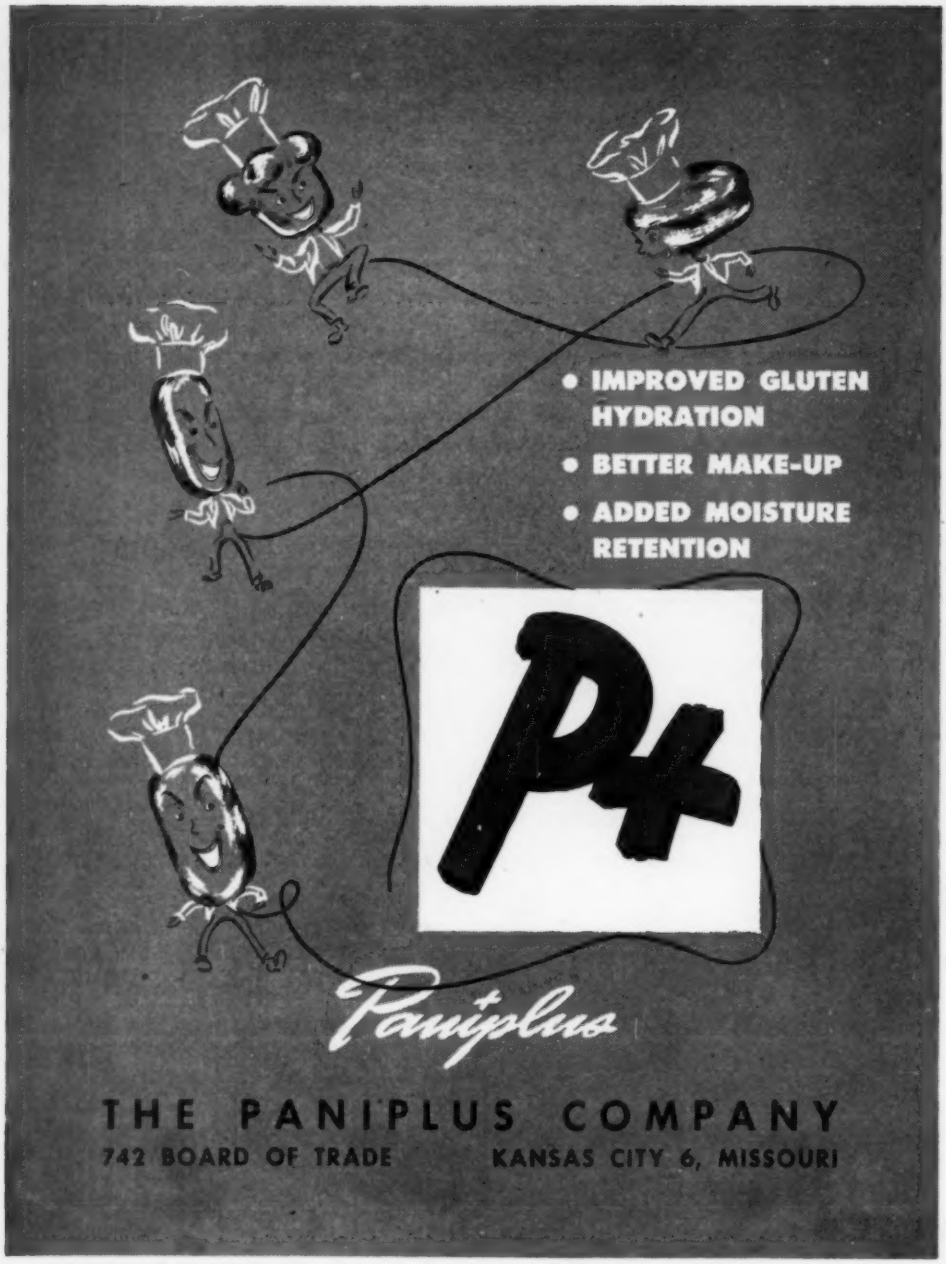
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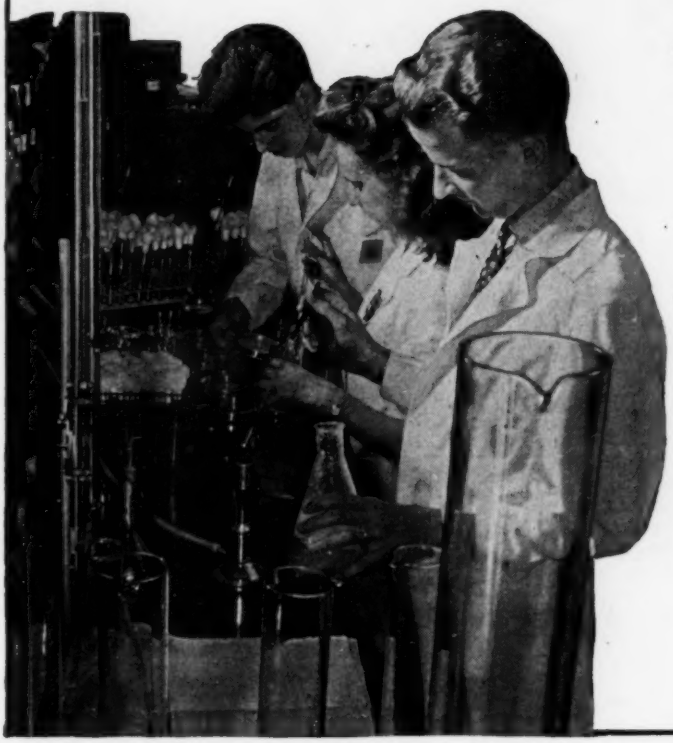

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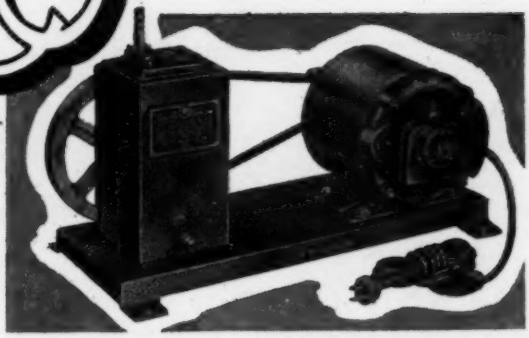

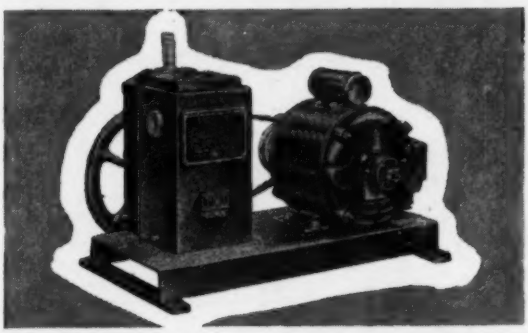

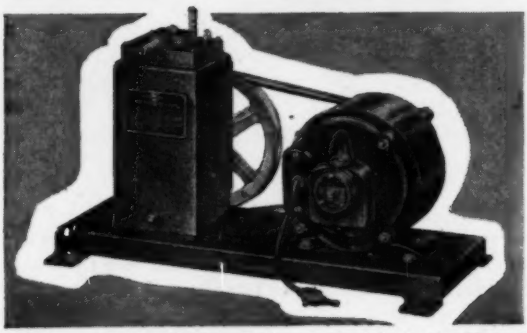

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CEREAL CHEMISTRY

VOL. XXIV SEPTEMBER, 1947 No. 5

COMPARATIVE BIOLOGICAL AVAILABILITIES OF VARIOUS FORMS OF IRON IN ENRICHED BREAD

HAROLD BLUMBERG and AARON ARNOLD

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(Presented at the Annual Meeting, May 1947; received for publication April 28, 1947)

The federal and state programs of flour and bread enrichment have made the biological availability of iron a subject of considerable importance to the milling, baking, and allied industries, as well as to the general public. Despite the importance of the problem, only a few studies have been published on the comparative biological values of the various forms of iron commonly used in bread enrichment. In order to be satisfactory for addition to dough mixes or to flour, an iron preparation must have no deleterious effect upon the quality of the bread or flour. Moreover, the iron should also be satisfactorily assimilable by the body.

Since the iron occurring naturally in wheat is considered to be associated largely with phytic acid, a phytate salt was one of the first types of iron suggested for bread enrichment. The iron of ferric phytate was reported by Andrews, Evans, and Huber (1941) to be utilized as well as saccharated iron oxide for hemoglobin formation when tested in iron-deficient anemic rats. Nakamura and Mitchell (1943) found that the utilization of ferric phytate in rats was comparatively poor, only about half that of the highly available ferric chloride. In human subjects, McCance, Edgecombe, and Widdowson (1943) observed that the presence in bread of sodium phytate, and to a less extent disodium phosphate, decreased the absorption of iron. Moore, Minnich, and Dubach (1943) found that ferric phytate, administered alone or in bread, was much less readily absorbed than ferrous sulfate both in normal human subjects and in patients with hypochromic microcytic anemia. Because of the relatively poor absorbability, these latter investigators concluded that ferric phytate was not a satisfactory form of iron for the enrichment of food.

Conflicting results have been reported in rat experiments on sodium iron pyrophosphate. Nakamura and Mitchell (1943) concluded that

it was as well utilized as reduced iron or ferric chloride. On the other hand, Street (1943) found sodium iron pyrophosphate to be a comparatively poor source of iron, less than 50% as effective as the highly available ferrous sulfate or ferric sulfate. Freeman and Burrill (1945) also reported sodium iron pyrophosphate to be poorly utilized, i.e., less than 50% as effective as ferric chloride. They found that sodium ferric orthophosphate, ferric orthophosphate, and reduced iron gave slightly lower values for availability than ferric chloride, but these differences were not significant.

The literature on this subject is not only incomplete, but also contains some important discrepancies. In view of this, the present investigation was undertaken to secure additional information on the comparative biological availabilities of various forms of iron in enriched bread, as determined by hemoglobin regeneration in rats made anemic from iron deficiency. The iron preparations were tested in the form of enriched breads baked with iron sources of current commercial interest, namely, ferrous sulfate ($FeSO_4$), ferric orthophosphate ($FePO_4$), reduced iron, and sodium iron (ferric) pyrophosphate ($Fe_4(P_2O_7)_3 \cdot 2Na_4P_2O_7 \cdot 6H_2O$).

Experimental Methods and Results

EXPERIMENT 1

Diets. The basal diet used in this experiment was patterned generally after that of Street (1943) and had the following percentage composition: dried bread 77, casein(low-iron) 12, salt mixture (low-iron) 3, and peanut oil 8. Supplements incorporated were, in mg per 100 g of diet: thiamine hydrochloride 1, riboflavin 2, pyridoxine hydrochloride 1, calcium pantothenate 4, niacinamide (nicotinamide) 2, choline chloride 100, inositol 100, copper (as $CuSO_4 \cdot 5H_2O$) 3, and manganese (as $MnSO_4 \cdot H_2O$) 1.5. Each rat received by stomach tube a weekly fat-soluble vitamin supplement equivalent to 2000 U.S.P. units of vitamin A, 400 U.S.P. units of calciferol (vitamin D_2), and 10 mg of α-tocopherol.

Since bread itself does not provide adequate protein, casein was included in the diet. The low-iron casein, which was prepared in the laboratory from skimmed milk, had an iron content of approximately 20μg per g. The low-iron salt mixture was prepared by modifying U.S.P. XI Salt Mixture No. 2. Ferric citrate was omitted. Since ample sodium chloride was supplied by the bread, sodium chloride also was omitted from the salt mixture and the amount of salt mixture was reduced from the usual 4% of the diet to 3%. Furthermore, the sodium biphosphate was replaced by potassium biphosphate.

All of the test breads were baked from the same lot of flour with special enrichment mixes that supplied the usual amounts of thiamine, riboflavin, and niacin, but varied with respect to iron. One lot of bread contained no added iron; the others were enriched by approximately 11 mg per lb (24.2 μg per g), dry weight, with the various forms of iron shown in Table I. This level of iron is within the range used in

TABLE I

IRON CONTENTS OF DIETS

Expt.	Group	Diet	No. rats	Iron content of bread	Iron content of diet
				μg/g	μg/g
1	1	Negative control bread (no added iron)	16	18.1	17.5
	2	Ferrous sulfate	15	41.9	35.8
	3	Ferric orthophosphate	15	40.7	34.9
	4	Reduced iron	15	38.0	32.8
	5	Sodium iron pyrophosphate	16	41.6	35.6
	6	Positive control (added iron 200 μg/g)	11	—	217.5
	7	Milk negative control	11	—	—
2	1	Negative control bread (no added iron)	17	11.8	14.2
	2	Ferrous sulfate	19	33.3	31.3
	3	Ferric orthophosphate	18	36.2[1]	31.1
	4	Reduced iron	19	32.9	31.0
	5	Sodium iron pyrophosphate	19	33.3	31.2
	6	Positive control (added iron 200 μg/g)	9	—	214.2

[1] Adjusted to 33.0 by slight dilution with negative control bread.

commercial enrichment. The iron samples themselves were analyzed and incorporated into the mix according to the iron content found. The reduced iron (U.S.P.) and ferric orthophosphate were from batches actually used for commercial enrichment; the ferrous sulfate was U.S.P. grade, and the sodium iron pyrophosphate was of a quality suitable for enrichment. After being air-dried at 37°C to a moisture content of approximately 4%, the breads were ground for use in the diets. Diet 6 was prepared by addition of ferrous sulfate to the negative control Diet 1 at a level of 200 μg of iron per g of diet. This provided a positive control to determine the maximum rate of hemoglobin regeneration permitted by the basal diet in the presence of a known optimal amount of available iron.

The actual iron contents of the various breads and diets are given in Table I. The iron analyses were made by a thiocyanate procedure (Eckert and Auerbach, 1944). The bread component contributed about 91% of the iron in the diet, 51% being from the enrichment mix and 40% from the iron present in the unenriched bread.

Animal Experimentation. Albino rats of the Sherman strain were prepared for iron-deficiency studies by special feeding precautions

generally similar to those described by Elvehjem and Kemmerer (1931). When weaned at about 24 days of age, the young rats were removed to new, individual, galvanized cages in which there was no exposed iron or rust. Anemia was induced in the animals by the feeding of certified cow's milk to which had been added cupric sulfate and manganous sulfate equivalent to 1.8 mg of copper and 1.4 mg of manganese per liter of milk. A few animals died during the depletion period. After 31 days of iron depletion, hemoglobin determinations were made on tail blood by the alkaline hematin method, as adapted for the Klett-Summerson colorimeter. Except for 11 somewhat resistant animals with hemoglobin values of 5–10 g per 100 ml, the rats were found to be sufficiently anemic for test purposes, i.e., had hemoglobin values of 2–5 g per 100 ml, with an average of about 2.9 g.

As shown in Table I, the animals were divided into seven groups of 11–16 rats each, with similar weight and sex distributions. The 11 less anemic rats formed Group 7, which was continued on the anemia-producing milk diet as another form of negative control. The experimental diets were then fed for four weeks, hemoglobin determinations and weighings being made at the end of each week.

Results. The hemoglobin regeneration curves are shown in Figure 1. Inasmuch as some of the animals in the highest test groups reached

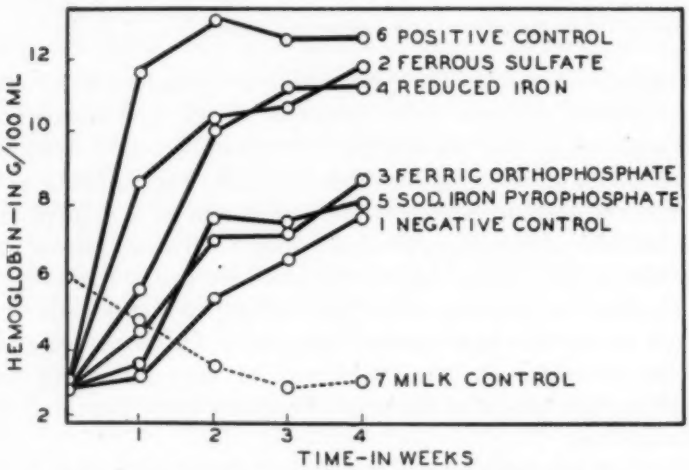

Fig. 1. (Experiment 1) Hemoglobin regeneration curves in anemic rats fed various iron source materials in form of enriched bread.

the normal range of hemoglobin values between the first and second weeks, the interpolated value for 1½ weeks appeared to be the most sensitive point for comparison. When some later point on the curve is used, the quantitative superiority of the more highly available forms

becomes somewhat masked because the animals that had already reached normal hemoglobin values by the second week were capable of little or no further gain. On the other hand, when the results at one week are used, the inferiority of the poorly available forms may be exaggerated due to an initial physiological lag in response, as evidenced by a sharp increase in the slope of the curve between the first and second weeks. The general trend of the results at 1½ weeks is confirmed by the curves in Figure 1 for other points during the four-week test.

The initial mean hemoglobin values, as listed in Table II, indicated that the four test groups were at approximately the same degree of anemia at the start of the experiment. Mean hemoglobin gains at 1½ weeks were calculated. However, since there were minor differences in the iron contents of the four test breads (cf. Table I), these mean values were corrected slightly on the basis of the iron content of Diet 2 (ferrous sulfate), which was highest in iron. The corrected means and standard errors are given in Table II. From the hemoglobin regeneration curves and the values for mean hemoglobin gains, it may be seen that the iron sources compared as follows, in order of decreasing availability: ferrous sulfate > reduced iron > ferric orthophosphate > sodium iron pyrophosphate.

The statistical significance of these differences was determined by calculation of the standard error of the difference between the mean hemoglobin gains at 1½ weeks, comparisons being made for all groups. A relatively high criterion of significance was adopted by basing conclusions only on P values of 0.01 or less (i.e., probability of difference being fortuitous equals 1 in 100, or less). As may be seen in Table II, the superiority of ferrous sulfate over ferric orthophosphate and sodium iron pyrophosphate was highly significant ($P = <0.001$), but its superiority over reduced iron was not quite significant ($P = 0.02$). Reduced iron also was significantly superior to ferric orthophosphate and to sodium iron pyrophosphate ($P = <0.001$). Ferric orthophosphate was not significantly superior to sodium iron pyrophosphate ($P = 0.62$).

The values for total hemoglobin gains at four weeks were also analyzed, although this is not a sensitive point for comparison. Ferrous sulfate was not significantly different from reduced iron ($P = 0.86$). However, even at this time both ferrous sulfate and reduced iron were still significantly superior to ferric orthophosphate and sodium iron pyrophosphate ($P = <0.001$), as shown in Table II. Ferric orthophosphate was not significantly superior to sodium iron pyrophosphate ($P = 0.46$).

The foregoing analysis established the order of superiority among the test forms of iron used in these experiments. For convenience,

TABLE II

HEMOGLOBIN REGENERATION AND GROWTH OF RATS ON DIFFERENT FORMS OF IRON

Expt.	Group	Diet	No. rats	Initial wt. (avg.)	Wt. gain (avg.)		Hemoglobin initial mean	Hemoglobin gain at 1½ weeks		Hemoglobin gain at 4 weeks		Availability
					1½ wks.	4 wks.		Mean ±S.E.	P[1]	Mean ±S.E.	P[1]	
				g	g	g	*g/100 ml*	*g/100 ml*		*g/100 ml*		%
1	1	Negative control bread	16	75	25	78	2.89	1.61±0.33	<0.001	4.89±0.40	<0.001	—
	2	Ferrous sulfate	15	70	32	89	2.85	6.55±0.31	—	8.98±0.40	—	100
	3	Ferric orthophosphate	15	73	25	82	2.89	3.04±0.44	<0.001	5.82±0.66	<0.001	29
	4	Reduced iron	15	75	33	91	2.88	5.38±0.36	0.02	9.09±0.49	0.86	76
	5	Sodium iron pyrophosphate	16	72	25	82	2.88	2.77±0.31	<0.001	5.24±0.40	<0.001	23
	6	Positive control	11	61	25	77	3.10	9.24±0.59	—	9.40±0.47	—	—
2	1	Negative control bread	17	72	30	81	4.31	0.72±0.26	<0.001	2.90±0.36	<0.001	—
	2	Ferrous sulfate	19	72	45	104	4.46	5.13±0.21	—	10.20±0.27	—	100
	3	Ferric orthophosphate	18	73	37	97	4.14	2.78±0.31	<0.001	6.68±0.33	<0.001	47
	4	Reduced iron	19	68	39	102	4.36	4.70±0.30	0.25	9.87±0.32	0.43	90
	5	Sodium iron pyrophosphate	19	73	38	98	4.35	1.54±0.28	<0.001	5.84±0.29	<0.001	19
	6	Positive control	9	66	42	112	2.88	9.64±0.27	—	12.90±0.28	—	—

[1] P = probability of fortuitous difference (from ferrous sulfate).

rather than for statistical accuracy, it was desirable to express the comparative availabilities quantitatively. This was done on the basis of mean hemoglobin gains at 1½ weeks. The effect due to the added iron was computed by subtracting from each value the amount of gain that resulted on the negative control diet alone. In Table II the availabilities of the types of iron are rated with reference to ferrous sulfate as 100%.

There were only slight differences in the weight gains of the four test groups, and these minor differences did not correlate with the large variations in hemoglobin regeneration (cf. Table II). An extra copper supplement failed to improve the hemoglobin regeneration in three additional rats fed the sodium iron pyrophosphate diet, thereby indicating that the poor response could not be attributed to an inadequacy of copper in the diet.

EXPERIMENT 2

Diets. For confirmation of the results secured in the first comparative test, a second experiment was conducted under closely similar conditions. New enrichment mixes were prepared. The second series of breads was enriched by approximately 10 mg of iron per lb (22 μg per g), dry weight, with the forms of iron shown in Table I. This level of iron was by intention slightly below the 11 mg per lb used in the first experiment, but still within the legal enrichment range. The samples were coded both in this laboratory and at the bakery, so that the identities of the breads were not known until after the conclusion of the experiment.

The basal diet differed from that of the previous experiment only in that the bread was increased from 77% to 82% at the expense of the peanut oil. Since the level of iron enrichment was lower in this series, it was considered safe to use the test component, bread, at its maximum proportion in the diet without introducing an amount of ferrous sulfate above the minimum for optimal response. The iron contents of the breads, as found by chemical analysis, are given in Table I. All of the iron-enriched samples were approximately 33 μg per g, dry weight, except Bread No. 3, which gave the slightly higher value of 36.2 μg per g. Therefore, Bread No. 3 was diluted slightly by addition of the negative control Bread No. 1 to give an iron content of 33 μg per g also. The bread component contributed about 87% of the dietary iron, 56% being from the enrichment mix, and 31% representing the iron present in the bread even when iron is omitted from the enrichment mix (e.g., Bread No. 1).

Animal Experimentation. Sherman strain albino rats were made anemic as previously described, although the anemia was not allowed

to become so severe as in Experiment 1. After 26 days of iron depletion, almost all of the rats had hemoglobin values of 2.5–5.0 g per 100 ml (average about 4.3 g), so that they were sufficiently anemic for test purposes. The animals were divided into six groups of 9–19 rats each, as shown in Table I. The experimental diets were then fed for four weeks, hemoglobin determinations and weighings being made at the end of each week. Group 1, the negative control, was continued for two additional weeks to determine how much time would be required to reach a hemoglobin value of 10 g per 100 ml, i.e., close to the beginning of the normal range.

Results. The general nature of the results is shown by the curves of hemoglobin regeneration in Figure 2. Mean hemoglobin gains at

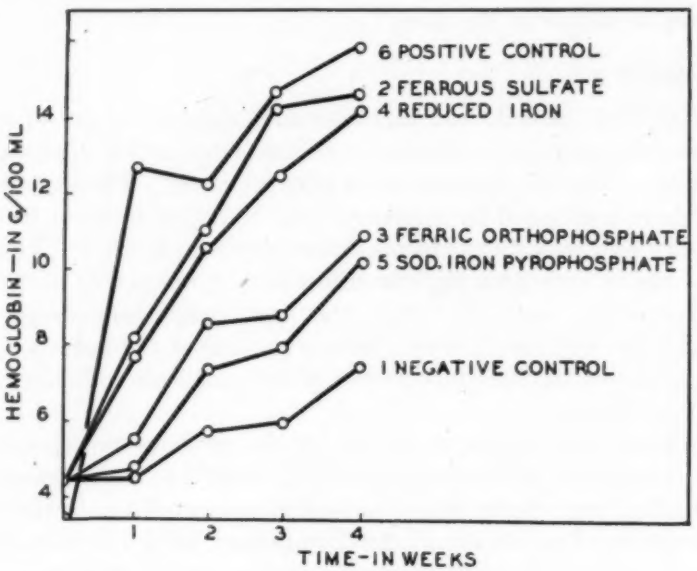

Fig. 2. (Experiment 2) Hemoglobin regeneration curves in anemic rats fed various iron source materials in form of enriched bread.

1½ weeks are given in Table II. The general trend of the results at this point is confirmed by the curves in Figure 2 for other points during the four-week test. In confirmation of the results of the first experiment, the iron sources again compared as follows, in order of decreasing availability: ferrous sulfate > reduced iron > ferric orthophosphate > sodium iron pyrophosphate.

The mean hemoglobin gains at 1½ weeks were analyzed statistically, with the previously mentioned P value of 0.01 or less as the criterion of significance. As may be seen in Table II, the superiority of ferrous sulfate over ferric orthophosphate and sodium iron pyrophos-

phate was again highly significant ($P = <0.001$), but its superiority over reduced iron was not significant ($P = 0.25$). Reduced iron was again significantly superior to ferric orthophosphate and sodium iron pyrophosphate ($P = <0.001$). In this second experiment the superiority of ferric orthophosphate over sodium iron pyrophosphate ($P = 0.005$) was slightly above the criterion of significance (apparently because the value at two weeks was unduly high with respect to the rest of the curve, as may be seen in Figure 2).

The values for total hemoglobin gains at four weeks were also analyzed, although, as mentioned previously, this is not a sensitive point for comparison. Ferrous sulfate was not significantly different from reduced iron ($P = 0.46$). However, at this point also the superiority of ferrous sulfate and of reduced iron over ferric orthophosphate and sodium iron pyrophosphate was highly significant ($P = <0.001$), as shown in Table II. At the four-week point, the superiority of ferric orthophosphate over sodium iron pyrophosphate was not significant ($P = 0.066$).

For an approximate quantitative comparison, the percentage availabilities were again calculated on the basis of the hemoglobin gains at 1½ weeks (cf. Table II). These figures were in general agreement with those of the first experiment. The various forms of iron were also compared on the basis of the number of days required to reach an average hemoglobin value of 10 g per 100 ml, which is close to the normal range (Table III). They were then rated for percentage

TABLE III

EXPERIMENT II. AVAILABILITIES OF FORMS OF IRON, BASED UPON DAYS REQUIRED TO REACH HEMOGLOBIN VALUE OF 10 G PER 100 ML

Group	Diet	Days	Availability (%)
2	Ferrous sulfate	11.9	100
4	Reduced iron	12.7	96
3	Ferric orthophosphate	24.8	46
5	Sodium iron pyrophosphate	27.7	34
1	Negative control bread	35.9	0

availability in comparison with ferrous sulfate. The results are in approximate agreement with the percentage availabilities found by the previous method (cf. Table II, Experiment 2).

In striking contrast to the wide variations in hemoglobin regeneration, the weight gains of the four test groups differed little. The differences in food consumption and iron intake were very small and could not account for the large differences in hemoglobin regeneration (cf. Table IV). As a matter of fact, even these small differences were

TABLE IV

EXPERIMENT II. HEMOGLOBIN GAINS, FOOD CONSUMPTIONS, AND IRON INTAKES OF RATS

Group	Diet	For 1½ weeks (avg.)			For 4 weeks (avg.)		
		Hemoglobin gain	Food consumption	Iron intake	Hemoglobin gain	Food consumption	Iron intake
		g/100 ml	*g/day*	*µg/day*	*g/100 ml*	*g/day*	*µg/day*
1	Negative control bread	0.72	9.4	134	2.90	9.2	131
2	Ferrous sulfate	5.13	11.6	363	10.20	12.4	388
3	Ferric orthophosphate	2.78	10.8	336	6.68	12.2	379
4	Reduced iron	4.70	11.3	350	9.87	12.5	387
5	Sodium iron pyrophosphate	1.54	10.9	341	5.84	12.2	382
6	Positive control	9.64	11.8	2528	12.90	12.9	2761

somewhat compensated for by the corresponding slight differences in growth. Furthermore, two rats each on the reduced iron diet and on the sodium iron pyrophosphate diet were paired-fed to maintain individual food consumption the same. The reduced iron group again showed the expected superiority.

For an over-all approximate comparison, the percentage availabilities in both experiments, as given in Table II, were averaged. The following results were obtained: ferrous sulfate 100, reduced iron 83, ferric orthophosphate 38, and sodium iron pyrophosphate 21.

Discussion

The experiments described in this report were designed to compare the iron preparations in a manner simulating as closely as possible the practical conditions of use in the enrichment program, i.e., actually baked in bread at the prescribed legal levels. The iron intake was at a normal or physiological level for rats, rather than in a therapeutic concentration. The results of the two experiments agreed closely; comparisons with the results of other investigators are made below.

Sodium iron pyrophosphate was poorly available for hemoglobin regeneration, as compared with ferrous sulfate. This is in agreement with Street (1943) and Freeman and Burrill (1945) and in disagreement with Nakamura and Mitchell (1943), who found sodium iron pyrophosphate highly available, i.e., equal to ferric chloride.

Ferric orthophosphate was also poorly available, being significantly inferior to both ferrous sulfate and reduced iron. In a single experiment Freeman and Burrill ranked ferric orthophosphate as slightly less effective than ferric chloride and slightly more effective than reduced iron, although their statistical treatment of the data indicates that these differences were not significant. The explanation for the discrepancy in results is not readily apparent. Since Freeman and Burrill give only the final hemoglobin values at the end of 28 days, a time at which the groups were already well within the normal range of hemoglobin values, it is possible that differences appearing at one to two weeks were no longer discernible. However, it may be pointed out that Day and Stein (1938) found ferric orthophosphate to be much less effective than ferric chloride in preventing a fall of hemoglobin in rats on an iron-deficient purified diet. Furthermore, the clinical studies of Moore, Arrowsmith, Welch, and Minnich (1939) indicated that in man the relatively insoluble ferric orthophosphate, as well as ferrous phosphate, was very poorly absorbed as compared with ferrous sulfate.

Reduced iron was found to be highly available, in agreement with Nakamura and Mitchell and with Freeman and Burrill. It is known to be efficacious clinically in iron therapy.

Ferrous sulfate was found to be highly available, of course, as is well known from its extensive clinical use as the customary form of iron in therapy.

Summary

The biological availabilities of various types of iron used for bread enrichment have been compared on the basis of hemoglobin regeneration in anemic rats. The iron preparations were fed in the form of enriched breads to groups of 15–19 rats each. The results of two experiments were substantially the same.

Under the conditions of these experiments, both ferrous sulfate and reduced iron were highly available. Ferrous sulfate gave slightly higher experimental values for availability than did reduced iron, but the differences were not statistically significant. However, both ferrous sulfate and reduced iron were significantly more available than ferric orthophosphate and sodium iron pyrophosphate. Ferric orthophosphate appeared slightly more available than sodium iron pyrophosphate, but this superiority was of questionable significance.

The biological availabilities of the various forms of iron were rated approximately as follows with reference to the highly available ferrous sulfate as 100%: ferrous sulfate 100, reduced iron 83, ferric orthophosphate 38, and sodium iron pyrophosphate 21.

These results demonstrate the differences in assimilability of various iron sources and serve to direct attention again to the desirability of using highly assimilable iron sources, such as ferrous sulfate and reduced iron, in bread and flour enrichment, so that the maximum benefit from the iron may be secured by the consumer.

Addendum

A more recent investigation at several widely spaced levels of enrichment designed to compare the dosage response curves has demonstrated that, under the conditions of the experiment, ferrous sulfate iron was approximately four to five times as effective as ferric orthophosphate iron in promoting hemoglobin regeneration in anemic rats. Details of the study will be reported later.

Acknowledgments

The authors are indebted to Mr. George Garnatz, of the Kroger Food Foundation, Cincinnati, Ohio, for the breads used in these experiments. The authors also wish to express their appreciation to Dr. M. L. Tainter and Dr. L. C. Miller for their interest and criticism, and to Mrs. Mary Lenz for statistical treatment of the data.

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PURIFICATION OF BARLEY MALT ALPHA-AMYLASE [1]

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Although the starch-degrading action of malt extract stands as a classic historical example of enzyme action, purification of the active principles of malt (alpha- and beta-amylase) has lagged behind similar purification studies on animal and yeast enzymes. Among the many factors contributing to this lag might be mentioned the relative instability of the amylases and, as will be shown later, the relatively small concentrations of these constituents in malt extracts in relation to both the total dry matter and to the total protein present. Furthermore, most early attempts at purification were directed towards the isolation of "malt amylase" preparations (Euler and Svanberg, 1921; Glimm and Sommer, 1927) which, were of course mixtures of alpha- and beta-amylase. More recently Caldwell and Doebbeling (1935) have reported an alpha-amylase preparation which "hydrolyzed 1000 times its weight of starch and formed 337 times its weight of maltose in 30 minutes at 40°." Myrbäck and Örtenblad (1941) reported a modification of the procedure of Holmbergh (1938) for preparation of alpha-amylase which resulted in a preparation with a specific activity (on a dry basis) about 20 times that of the starting malt extract. Kneen, Sandstedt, and Hollenbeck (1943) have demonstrated that calcium ions are necessary for the stability of alpha-amylase. They have also presented data on ammonium sulfate and alcohol fractionation, and also on the heat stability of the malt amylases, which indicated the probable utility of these procedures in purification.

The present communication describes procedures for purifying malt alpha-amylase that have been developed by following the protein nitrogen concentrations and enzyme activities of the various fractions. The most highly purified preparations obtained contained about 60% of the activity originally present in the malt extract, and less than 0.5% of the original protein nitrogen. After drying, 1 mg of this preparation is as active (with respect to alpha-amylase) as 5 g of malt.

[1] Enzyme Research Laboratory Contribution No. 106.

Materials and Methods

Malt Samples Used. Although a wide variety of malts was used for preliminary work, almost all of the results reported here were obtained with a 100-pound sample of commercial malt containing 7.8% moisture and 2.16% nitrogen (dry basis). The "Maltose Equivalent" of this malt (four times the Lintner value) was equal to 893; the beta-amylase equivalent was 826; and the alpha-amylase equivalent was 67.

TABLE I

PURIFICATION OF MALT ALPHA-AMYLASE: PROCEDURE A

Step No.	Procedure	Total volume ml	Total units $\times 10^{-6}$	Specific activity	
				Units per mg dry matter (Maltose Equivalent)	Units per mg protein—protein (N×6.25)
1	2,000 g ground malt + 15 L 0.1% $CaCl_2$, adjust to pH 6.0 with NaOH, stir 2 hours *Suspension*	15,500	125	67	510
2	Squeeze suspension through cheesecloth; centrifuge extract, discard residue *Supernatant*	12,500	105	220	2,050
3	Heat supernatant at 70° for 15 minutes, cool to room temperature, filter through layer of 50 g diatomaceous earth (Celite), discard residue *Filtrate*	12,300	103	300	11,500
4	Ammonium sulfate fractionation; retain fraction between 0.22 and 0.45 saturated, take up residue in 0.1% $CaCl_2$ *Solution*	1,000	90	—	46,000
5	Add 25 g bentonite, stir 1 hour, centrifuge, discard residue *Filtrate*	890	75	—	88,000
6	Ammonium sulfate fractionation; retain fraction between 0.1 and 0.35 saturation, take up residue in 0.1% $CaCl_2$ *Solution*	75	70	75,000	105,000
	Repeat steps 5 and 6 *Solution*	50	60	103,000	163,000

Determination of Amylase Activities. When beta-amylase was present, both alpha- and beta-amylase were determined by the methods of Olson, Evans, and Dickson (1944). After the beta-amylase of the malt was destroyed by heating (see Table I, step 3) the alpha-amylase was assayed by a more convenient procedure. To 10 ml of a 2%

buffered solution of soluble starch (0.05 *M* acetate, pH 4.7) were added 5 ml of solution containing 0.5 ml 0.1 *M* calcium chloride solution, enzyme, and water. After 5, 10, or 20 minutes at 30°, depending upon the rapidity of the starch breakdown, 0.5 ml of the digestion mixture was added to 5 ml of a solution of iodine in potassium iodide (0.0035 *M* with respect to iodine and 0.25 *M* with respect to potassium iodide). This hydrolyzate-iodide solution was then diluted by the addition of 10 ml of water, and the light transmission of the diluted mixture at 660 mμ was measured in an Evelyn photoelectric colorimeter at 25°. The relation between percentage transmission and units of alpha-amylase is shown in Figure 1.

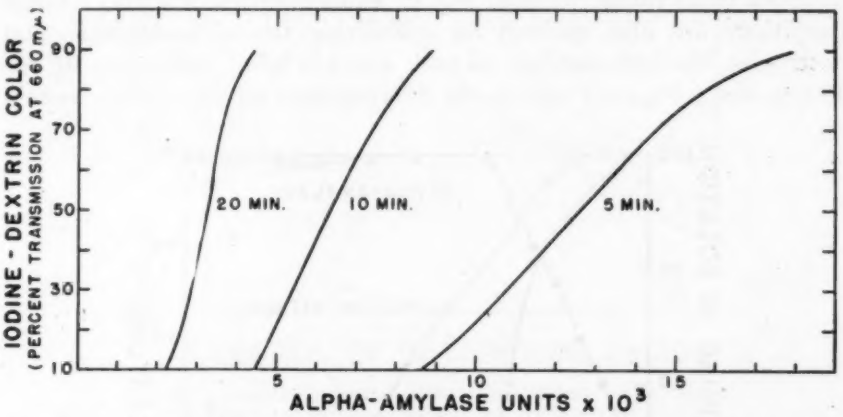

Fig. 1. Relationship between units of alpha-amylase and color change of the dextrin—I_2 complex after 5, 10, and 20 minutes incubation, using increasing concentrations of the enzyme with soluble starch under the conditions described in the text.

Expression of Amylase Units. The units used in this report describe the amount of alpha-amylase present in the digestion mixture and do not refer to any particular volume or weight of material containing the enzyme. Numerically the units are those employed by Olson, Evans, and Dickson (1944) transformed from a "specific" activity (Maltose Equivalent) to activities which do not in themselves infer the weight of the enzyme preparation. These amylase units per mg of dry weight, as described by Schwimmer (1947), are numerically equal to the "Maltose Equivalent."

Other Methods Used. Phosphatase was determined by the liberation of nitrophenol from nitrophenyl phosphate (Axelrod, 1947); phosphorylase by the liberation of phosphate from glucose-*l*-phosphate by the method of Green and Stumpf (1942); maltase by the method of Schwimmer (1945); proteinase by liberation of tyrosine at pH 6, a modification of the method of Anson (1937); and peroxidase by the delayed oxidation of HI (Schwimmer, 1944). Protein was estimated

by determining the nitrogen content (by micro-Kjeldahl technique) of proteins of the various preparations after precipitation of the proteins by trichloroacetic acid and washing of the precipitate to remove non-protein nitrogen. The reducing action of dextrins and sugars was determined by use of the Somogyi sugar reagent (1945).

Results

Extraction and Heating. Kneen, Sandstedt, and Hollenbeck (1943) have shown that alpha-amylase can be extracted most thoroughly at pH 6.0 in the presence of calcium salts. It has been found that, under aseptic conditions, malt extracts can be kept for two weeks at room temperature without loss of alpha-amylase activity. These conditions are also optimal for stabilizing the alpha-amylase and destroying the beta-amylase of malt extracts when heated to 70° for 15 minutes. Figure 2 depicts the effect of heat on the soluble protein

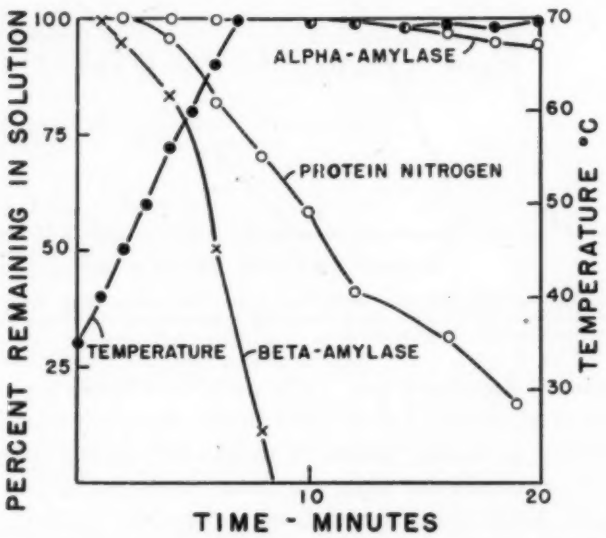

Fig. 2. Changes in protein nitrogen, alpha-amylase, and beta-amylase of a malt extract upon heating. The rise in temperature with time is shown by the temperature curve (referring to the right-hand ordinate); the losses in beta-amylase and protein nitrogen are measured on the left ordinate.

nitrogen and on the alpha- and beta-amylase activities of a malt extract prepared according to steps 1 and 2 of Table I. This heating step not only destroys all of the beta-amylase present in the malt extract but also removes between 75 and 85% of the total protein. It has been observed that when extracts of various malts of equal alpha-amylase and nitrogen contents are heated, those malt extracts with higher beta-amylase values usually precipitate a greater portion of protein than those of low beta-amylase content.

Ammonium Sulfate Fractionation. It has been found that the alpha-amylase of malt extracts could be completely precipitated at 0.5 saturation with ammonium sulfate (Caldwell and Doebbeling, 1935; Kneen, Sandstedt, and Hollenbeck, 1943). Figure 3 shows the

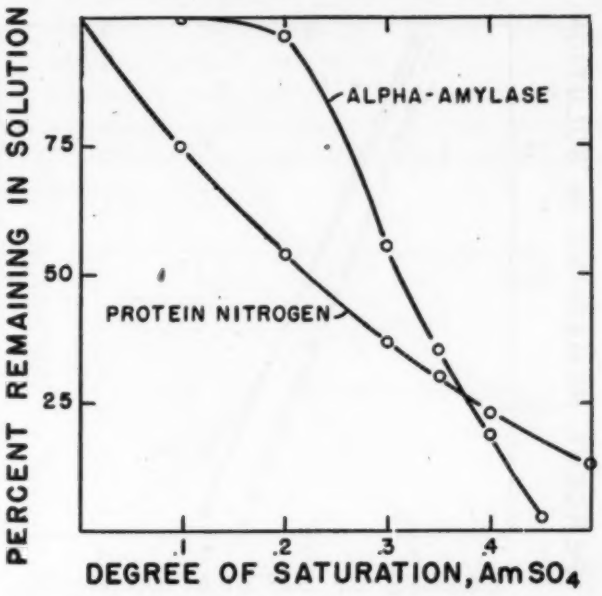

Fig. 3. Effect of adding ammonium sulfate to heated malt extract—protein nitrogen and the alpha-amylase remaining in solution.

percentage of alpha-amylase and protein nitrogen remaining in solution after adding increasing amounts of ammonium sulfate to a heated malt extract. This method of presentation is useful in deciding whether or not a particular fractionation procedure is of any value. Thus, Figure 3 readily shows that if the fractionation is so performed that the upper and lower 5% of the enzyme are discarded, then 70% of the protein will be eliminated, resulting in a threefold increase in specific activity on a protein basis. Further fractionation of this solution is of no obvious advantage since it does not affect the specific activity favorably, as may be seen from Figure 4. However, ammonium sulfate fractionation can be used to advantage at a later stage in the purification (Table I, step 6). The acidity of the protein suspensions varied between pH 5.6 and pH 5.8.

If the freshly prepared malt extract is not treated immediately, but is allowed to remain at room temperature for seven days in the presence of toluene and thymol, the fractionation characteristics with ammonium sulfate are significantly altered. The enzyme is not fully precipitated until 0.65 saturation and the protein precipitation parallels that of the

enzyme rather closely. This change in fractionation properties upon autolysis can possibly be attributed to the change of protein constituents due to proteolysis occurring during the incubation at room temperature.

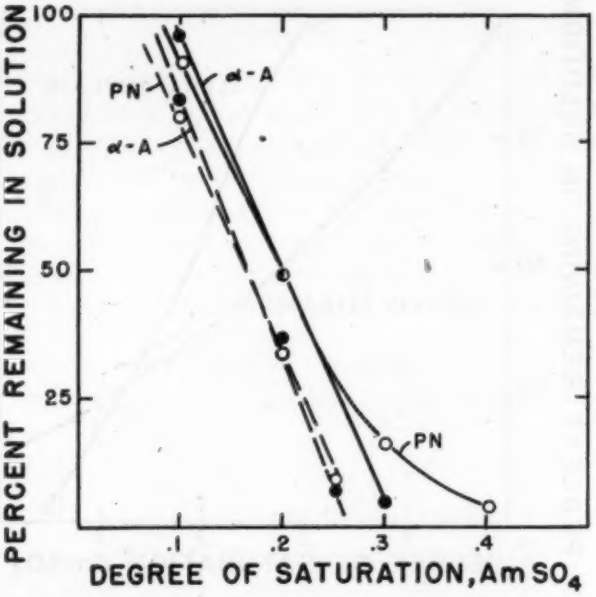

Fig. 4. Protein nitrogen PN (open circles) and alpha-amylase (closed circles) remaining in solution after the addition of ammonium sulfate to (*a*) once fractionated and heated malt extract (unbroken lines) and (*b*) twice fractionated and heated malt extract (broken lines).

Fractionation by Adsorption with Bentonite. When bentonite (Volclay) was added in increasing amounts to a series of aliquots of the fraction obtained by ammonium sulfate fractionation (Table I, step 4), an appreciable portion of protein and colored impurities could be adsorbed on the bentonite, leaving behind a lightly colored, highly purified solution of alpha-amylase (Figure 5). The adsorption on bentonite is a time reaction, and the success of this step depends upon not allowing the bentonite to stand too long in contact with the solution. It was also found that the adsorption of both protein and enzyme is more complete at lower pHs.

When bentonite was added in a similar manner to the preparations obtained by ammonium sulfate precipitation of heated and incubated malt extracts (Procedure B), no decidedly advantageous increase in specific activity could be obtained. However, if a similar malt extract was treated with bentonite at a concentration of 0.25%, enough protein impurities were adsorbed to increase the specific activity twofold (Table II, step 4). Furthermore the unadsorbed enzyme could now

be adsorbed by adding further amounts of bentonite and then, subsequently, could be eluted from the bentonite with a 5% pyridine solution containing 0.1% $CaCl_2$.

Use of Other Fractionation Procedures. During the course of these investigations, many fractionation procedures were tried, but no one was entirely satisfactory. Precipitation with lead acetate or sodium chloride gave variable results. The use of organic solvents and of acid pHs, while precipitating much protein, rendered the enzyme extremely unstable. Use of nucleic acid (Butler, 1945), kaolin (Sabalitschka and Weidlich, 1929) and starch granules (Holmbergh, 1933) as adsorbents proved unsatisfactory.

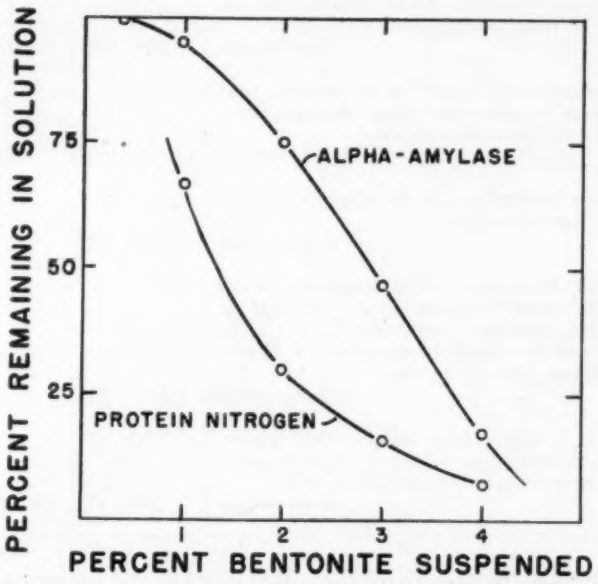

Fig. 5. Adsorption of protein and alpha-amylase by increasing concentrations of bentonite added to malt extract previously heated and fractionated with ammonium sulfate. The volume of this fraction is 1/15 that of the original malt extract. Percent bentonite is equal to grams bentonite suspended in 100 cc of solution.

Properties of Final Preparations. The two procedures adopted as standard are summarized in Tables I and II. Method A, which preceded Method B chronologically, gave a preparation which was light brown in color, gave a positive Molisch test for carbohydrate, and contained 10.6% protein nitrogen (64% protein). This preparation, which exhibited reducing action when tested with Somogyi's copper reagents (1945), increased its reducing action three- to fourfold when hydrolyzed with 3 *N* HCl. This may indicate the presence of reducing dextrins of low molecular weight in association with the protein. These dextrins could be eliminated by allowing the malt extract to

TABLE II

PURIFICATION OF MALT ALPHA-AMYLASE: PROCEDURE B

Step No.	Procedure	Total volume ml.	Total units $\times 10^{-4}$	Specific activity	
				Units per mg dry matter (Maltose Equivalent)	Units per mg protein—protein (N ×6.25)
1	2,000 g ground malt + 15 L 0.1% $CaCl_2$, adjust to pH 6.0 with NaOH, stir 2 hours *Suspension*	15,500	125	67	510
2	Squeeze suspension through cheese cloth, centrifuge extract. Allow supernatant to stand at room temperature for 7 days *Supernatant*	12,500	105	220	2,230
3	Heat supernatant at 70° for 15 minutes; cool to room temperature, filter through layer of 50 g diatomaceous earth *Filtrate*	12,300	104	310	13,600
4	Add 30 g bentonite, stir 30 minutes, centrifuge, discard residue *Supernatant*	12,000	90	—	29,100
5	Add 30 g bentonite, stir 30 minutes. Centrifuge, discard supernatant. Residue + 1 L 5% pyridine containing 0.1% $CaCl_2$; let stand at room temperature 2 hours, centrifuge, discard residue *Supernatant*	1,000	81	—	85,000
6	Add 225 g ammonium sulfate (0.37 saturated), centrifuge, discard supernatant; take up residue in 200 ml 0.1% $CaCl_2$ *Supernatant*	200	71	356,000	360,000

autolyze at room temperature for seven days in the presence of thymol and toluene. Such autolysis probably allowed both the alpha- and beta-amylase to completely hydrolyze any soluble dextrin and starch present in the extract. At any rate the pure preparations made by Procedure B contained between 16 and 16.5% nitrogen and gave negative or very doubtful positive Molisch tests. These preparations were somewhat more highly colored than those made by Method A. Their light absorption was determined by means of a Beckman spectrophotometer. There were no characteristic absorption peaks throughout the visible spectrum. The usual absorption by protein at 280 mμ was partially masked by a general absorption probably due to the highly colored impurities present (Figure 6).

The enzymic properties of the preparations corresponded to those generally attributed to malt alpha-amylase. The enzyme did not

dialyze through cellophane, and was inactivated and rendered insoluble when dialyzed against distilled water. This inactivation could be prevented by either dialyzing against 0.05% calcium chloride solution or by placing an excess of calcium sulfate in the dialysis bag. Repeated precipitation with ammonium sulfate also resulted in a water-insoluble and inactive material unless calcium chloride solution

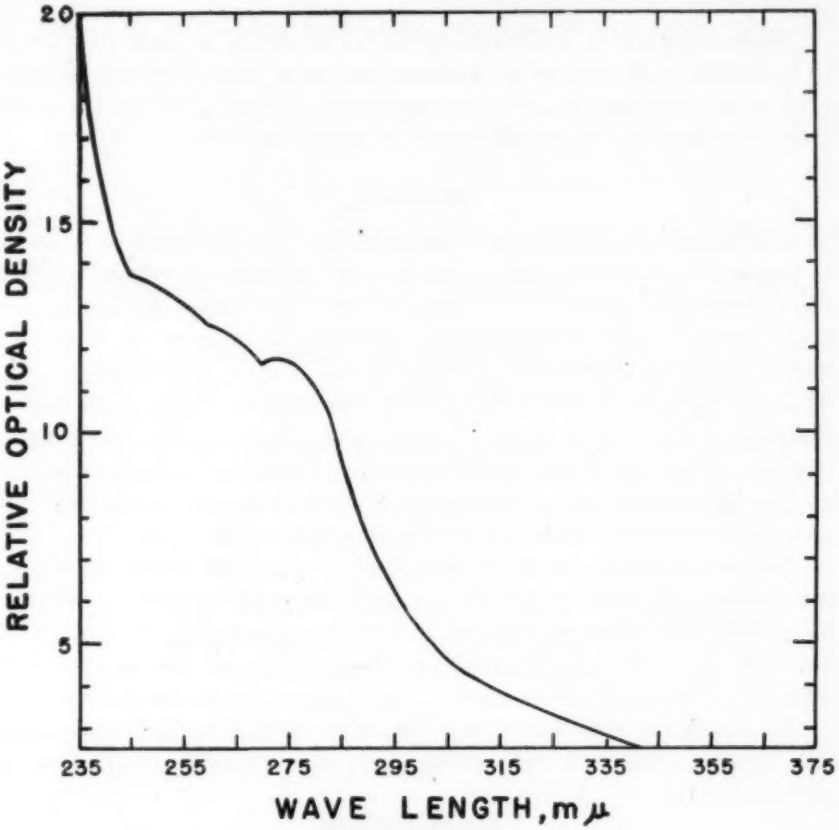

Fig. 6. Ultraviolet absorption spectrum of Preparation B6.

was used to dissolve the precipitates. Sodium tetraphosphate ($Na_6P_4O_{13}$) inactivated the enzyme when incubated at pH 5.0 in concentrations high enough to tie up the calcium in the preparations. Sodium phosphate under the same conditions of acidity and temperature did not affect the alpha-amylase activity. The dependence of the amylase stability on the presence of calcium ion is in accordance with the observations of Kneen, Sandstedt, and Hollenbeck (1943). In agreement with the observation of Caldwell and Doebbeling (1935),

the purified enzyme is much more sensitive to heat than when present in the crude extract.

The final preparation (B6) contained 67% of the total alpha-amylase of the malt extract and 0.4% of the original extract protein. If the protein in this preparation represents only pure enzyme protein, the maximum concentration of alpha-amylase in the malt extract cannot amount to more than 0.06% of the dry weight nor more than 0.6% of the protein of the extract.

Alpha-amylase preparations prepared by either method were found to be devoid of the following enzyme activities: beta-amylase, maltase, proteinase, peroxidase, and phosphorylase. They contained a maximum of 0.02% of the original acid phosphatase.

Summary

Purification studies on the alpha-amylase of malt extracts have led to preparations which contain 60–70% of the alpha-amylase, 0.4% of the protein, and about 0.05–0.08% of the dry matter of the starting malt extract. This amounts to a 150-fold purification on a protein basis and about 1,500-fold purification on a dry weight basis. The Maltose Equivalent (4 × °L) was about 300,000. One milligram of the preparation is as active as 5 g of malt. Each possible purification step attempted was followed by estimating the protein nitrogen as well as the alpha-amylase content of the various fractions obtained by varying the concentration of the fractionating agent used.

The purification procedure which has yielded the most potent preparations consists (in brief) of: autolysis of the malt extract, followed by adsorption of impurities on bentonite; adsorption of the alpha-amylase by further addition of bentonite; elution of the enzyme with pyridine solution; fractionation of the eluate with ammonium sulfate.

The stability and hydrolytic properties of the purified alpha-amylase are in agreement with those properties generally attributed to alpha-amylase.

Acknowledgments

Grateful acknowledgment is made to Dr. A. K. Balls of the Enzyme Research Laboratory for helpful suggestions during the course of this work. The author also wishes to thank G. F. Bailey of the Western Regional Research Laboratory for the ultraviolet absorption data and Dr. A. Frieden of the Pabst Brewing Company of Milwaukee for his generous gift of barley malt.

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AMYLASE ACTIVITY OF THREE BARLEY VARIETIES AS INFLUENCED BY DIFFERENT MALTING CONDITIONS[1]

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This report represents a continuation of previous studies of the changes taking place in the barley kernel during the malting process (Shands *et al.*, 1941; Shands *et al.*, 1942; and Dickson and Burkhart, 1942). These earlier studies did not include data on the influence of malting conditions upon the development of the individual amylase components. Therefore, emphasis is given here to the formation of alpha- and beta-amylase in three samples of barley malted under different conditions of moisture, time, and temperature.

[1] Based on cooperative investigations between the Division of Cereal Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, A.R.A., U.S.D.A., Wisconsin Agricultural Experiment Station, United States Maltsters Association, and Malt Research Institute.

Competent reviews of the literature on the effects of various factors upon the development of the amylases in the course of germination of cereals are given by Weichherz and Asmus (1931), Lüers (1936), and more recently by Kneen, Miller, and Sandstedt (1942). Anderson and Sallans (1938), Anderson and Meredith (1938), and Sallans and Anderson (1939) in studies of varietal differences of barley have reported on the influence of moisture, temperature, and germination time on diastatic power.

In earlier studies by Shands *et al.* (1941) using constant malting temperatures, and Shands *et al.* (1942) using systematically changed temperatures during malting, it was shown that several quality factors were affected besides the diastatic enzyme system of barley. Reference should be made to these papers for information relating to the other quality factors.

Shands *et al.* (1941) found a close relationship between factors influencing acrospire growth and diastatic power. They also reported that high malting moisture and increased malting time favored the development of diastatic power. The increase in diastase was greatest in the early phases of malting, and was less from 6 to 8 days under most of the conditions employed. Although all varieties studied showed a common trend, the magnitude of the changes differed between varieties. In later studies (1942), these workers found the maximum average diastatic power was obtained in a temperature change schedule during malting involving 12° and 16°C and 6 days total germination. Moreover, it was found that higher diastatic power usually accompanied higher wort and formol nitrogen ratios, and shorter conversion times.

In studies relating to the distribution of diastatic power in the barley and malt kernel, Dickson and Burkhart (1942) found the bulk of the increased activity was formed between the second and sixth day of malting. Higher malting moisture (48%) gave uniformly higher values than did the lower moisture (43%) samples. Increase in activity occurred only in the germ portion of the kernel, the distal half remaining essentially unchanged after the fourth day of malting. The results of these workers on alpha-amylase development were essentially the same as those reported for diastatic power.

Methods and Materials

Samples of Wisconsin Barbless (C.I. 5105) from Michigan, Wisconsin hybrid H35-7-2-1-3 (C.I. 7115) grown at Madison, Wisconsin, and Peatland (C.I. 5267) obtained from St. Paul, Minnesota, all grown in the 1943 crop year, were chosen for this study. The Wisconsin Barbless was low, Wisconsin H35-7-2-1-3 intermediate, and Peatland

high in amylase activity. The nitrogen contents of the barleys used were: Wisconsin Barbless, 1.92%; Wis. H35-7-2-1-3, 2.32%; and Peatland, 2.61%. Since these barleys were chosen for their ranges in amylase values, the responses of the samples to malting conditions cannot be attributed solely to variety.

The barleys were cleaned, steeped in water at 16°C in screen-bottomed cans 5 inches in diameter and 3 inches high to either 45%, or 48% moisture, and the steeped barleys germinated for 4, 6, 8, or 10 days in the small experimental unit previously described by Shands *et al.* (1942) at 12°C, 16°C, 20°C, or under the conditions of temperature change employed, i.e., 2 days at 20°C and the remaining 8 days at 12°C. At the end of the indicated germination times, the green malts were subjected to a uniform kilning procedure, namely: 8 hours at 25°C, 4 hours at 35°C, and 20 hours at 45°C. The kiln used by Shands *et al.* (1941) was employed in the drying of these malts. The dried samples (about 6.3% moisture) were freed of rootlets, and the cleaned malts were analyzed by standard methods.

Diastatic activity was determined by the alkaline ferricyanide method described in *Cereal Laboratory Methods*, A.A.C.C., 1941. Alpha-amylase was determined by the method of Sandstedt, Kneen, and Blish (1939) as modified for 20°C by Olson, Evans, and Dickson (1944). The activity is expressed in 20°C dextrinizing units as described by these authors in a recent communication (1947). Beta-amylase was calculated from the alpha-dextrinizing activity[2] and the diastatic activity in the customary manner (Olson, Evans, and Dickson, 1944).

In addition to the amylolytic enzyme values, extract, wort, and formol nitrogen, kernel weight, growth, and other values reported by Shands *et al.* (1941, 1942) were obtained. These values are not discussed in detail here, inasmuch as the effects of the various treatments are in agreement with those reported previously.

Results

The four variants, time, temperature, moisture, and sample malted, affected the amylase content of the malts produced. The influence of each of these on the amylase contents of the malts will be discussed. The results of the amylase determinations made upon the malts produced as indicated above are presented in Table I and in Figures 1 to 3.

Effect of Malting Conditions on Diastatic Power. The influence of germination conditions on the diastatic power of the malts produced in this study is represented graphically in Figure 1. The higher (48%)

[2] The terms "alpha-amylase" and the more strictly correct "alpha-dextrinizing activity" will be used interchangeably in this discussion.

malting moisture usually resulted in increased diastatic power in the three barley samples studied. Malts produced from Peatland barley at 48% moisture under the conditions of temperature change during germination showed lower diastatic activity than those malted under

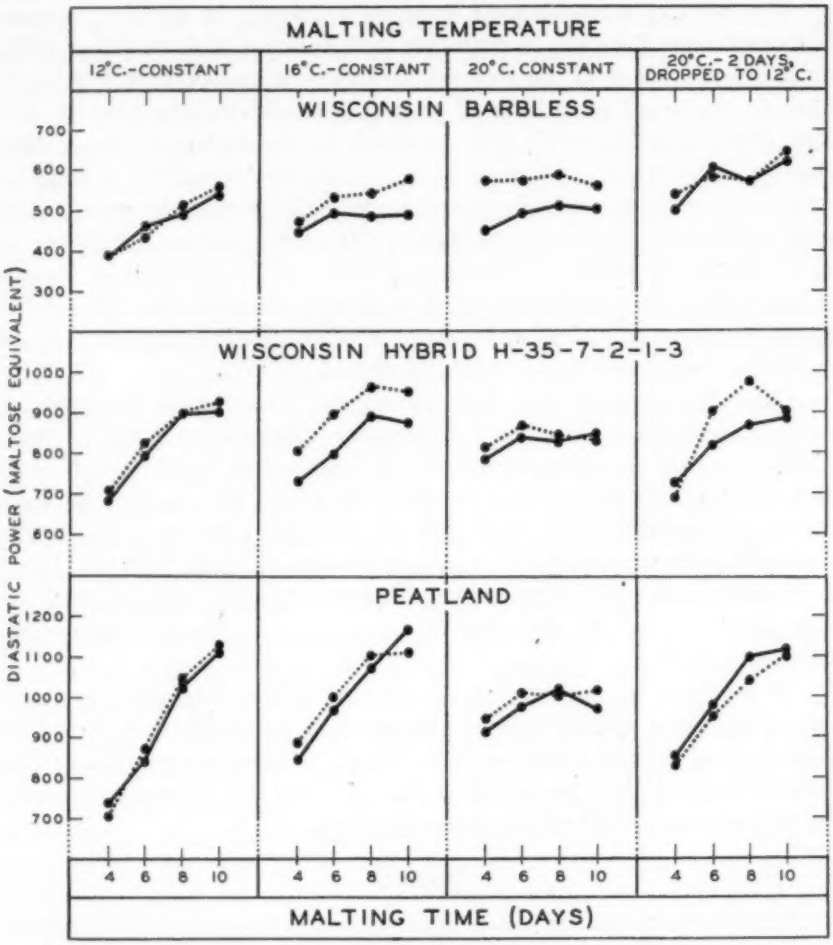

Fig. 1. The effect of malting time and temperature upon diastatic power of malts produced at 45% (————) and 48% (.....) moisture.

the same conditions at 45% moisture. The influence of moisture upon diastatic power was not as great at the 12°C as at the 16°C malting temperature. Germination at 48% malting moisture and 20°C resulted in malts of higher diastatic activity in the Wisconsin Barbless malts; this effect of moisture at this temperature was not as apparent in the other two samples studied.

A general increase in diastatic activity with increased malting time through the eighth day of germination was apparent in the malts produced at 12°C, 16°C, and in the temperature change series. The changes in diastatic activity from the eighth to tenth day of germination were unpredictable, and the increase or decrease in activity was dependent on sample malted and temperature. However, Peatland and Wisconsin Barbless germinated at 12°C showed significant increases with time. Peatland diastase also increased between 8 and 10 days at 45% moisture and 16°C. The bulk of the diastatic power in the malts germinated at 20°C apparently had been formed in the first 6 days of germination; the changes in activity thereafter were slight.

The diastatic activity of the 4-day malts increased as the temperature of malting was raised through 20°C, while the activity of the 4-day malts germinated under the temperature change conditions simulated those produced at either 12°C, or 16°C. Final diastatic power values were usually lower when the barleys were malted at 20°C than they were at the other temperatures. Malting at 12°C and under the temperature change schedule yielded malts of approximately equal final diastatic activity.

Effect of Malting Conditions on Beta-Amylase. The statements made above on the influence of malting conditions upon diastatic activity apply to beta-amylase development, as can be seen in Figure 2. The values are somewhat lower because alpha-amylase saccharifying activity has been removed from the diastatic power values. However, a few observations can be made which were masked in the diastatic activity measurements. The final level (10-day malt) of beta-amylase decreased with an increase in malting temperature in the Wisconsin hybrid H35-7-2-1-3, and Peatland malts, if averages of the two moisture levels are considered. There is little effect of malting temperature upon the final value of beta-amylase in the Wisconsin Barbless malts, except that the high moisture malts had higher beta-amylase than those produced under the low moisture conditions. A loss of beta-amylase activity at 20°C and high moisture was observed in the Wisconsin hybrid H35-7-2-1-3 and Peatland malts following the sixth day of germination, and in the Wisconsin Barbless malts following the eighth day of germination at 20°C. At 16°C, the Wisconsin hybrid malts showed a decrease in beta-amylase from 8 to 10 days growth.

It should be pointed out here that the loss of beta-amylase activity is accentuated somewhat when the results are calculated to the basis of the original barley used (including the dry matter lost in respiration, cleaning, etc.). However, the trends presented in this report would not be altered materially by the recalculation.

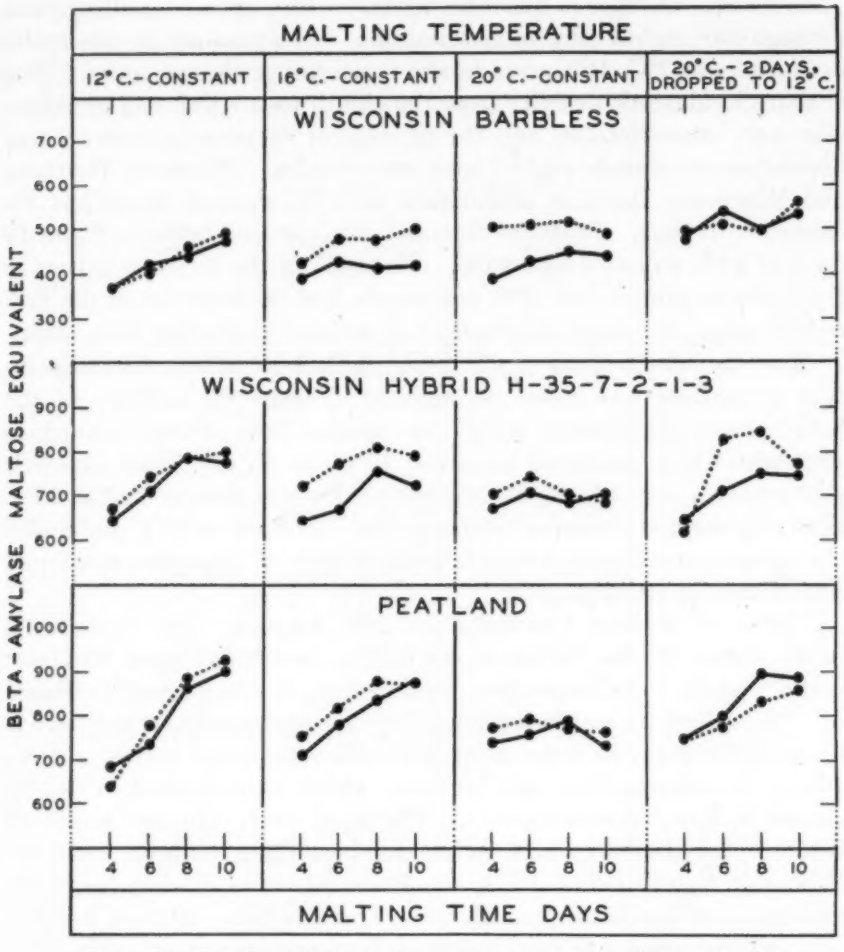

Fig. 2. The effect of malting time and temperature upon beta-amylase activity of malts produced at 45% (————) and 48% (.....) moisture.

Effect of Malting Conditions on Alpha-Dextrinizing Activity. The development of alpha-dextrinizing activity in the course of germination under the experimental conditions employed is presented in Figure 3. Malting moisture seemed to have little, if any, effect upon alpha-amylase development in this experiment.

There was a general increase in alpha-amylase with longer germination times, and the rate of increase was less at the higher temperatures. There was a marked increase in alpha-amylase between 4 and 8 days growth in H35-7-2-1-3 and Peatland malts. At the 4 and 6 day stages of germination, an increase in malting temperature resulted in increased alpha-dextrinizing activity in the Wisconsin hybrid H35-7-2-1-3

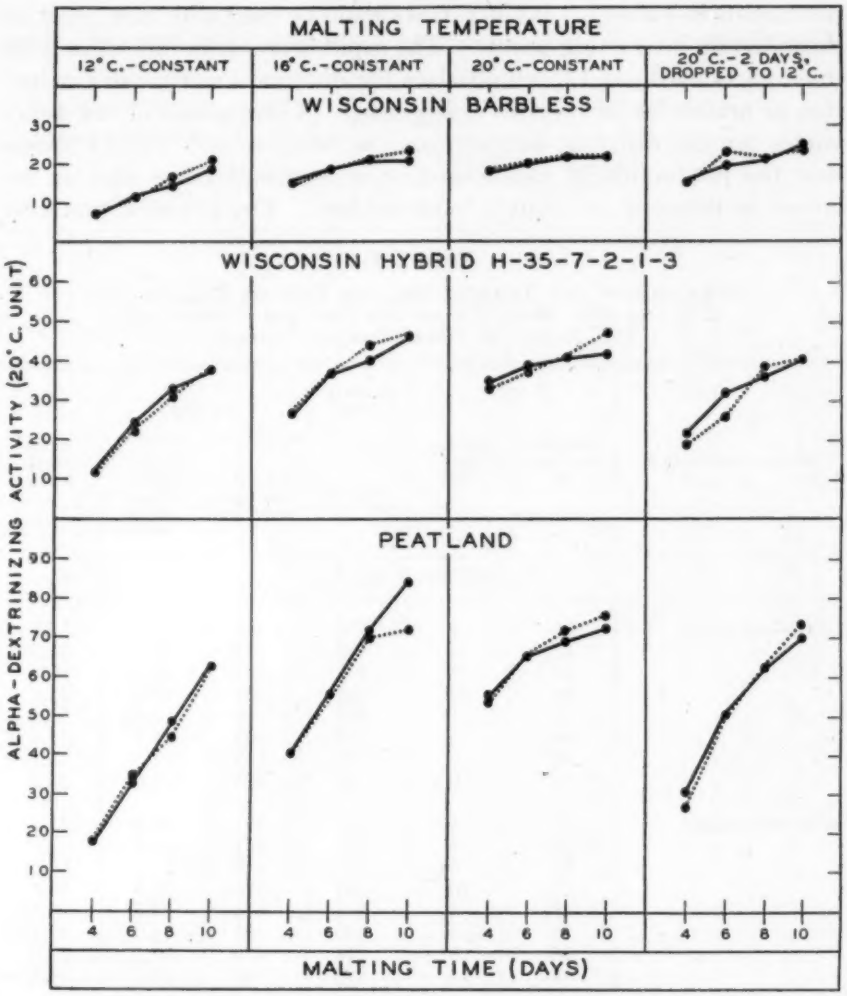

Fig. 3. The effect of malting time and temperature upon alpha-dextrinizing activity of malts produced at 45% (———) and 48% (.....) moisture.

and Peatland malts. There was little effect of malting temperature above 16°C upon the 8 and 10-day malts except for Peatland at 45% moisture. The 12°C malts were lower in activity throughout. As might be expected, malts produced by the temperature change schedule assumed a position intermediate between the 12°C and 16°C series for Wisconsin hybrid H35-7-2-1-3 and Peatland, but the Wisconsin Barbless malts produced under these conditions had activities similar to those malted at 16°C.

Effect of Malting Conditions on the Beta-Amylase Alpha-Amylase Ratio. The ratios of beta- to alpha-amylase are presented for all

treatments in Table I. While ratios should be used with care, they do show certain interesting trends. The rapid decrease in this value with time, particularly at 12°, emphasizes the difference in time of production or activation of the two components. Comparison of the 4-day values for the different temperatures within any one variety shows that the production of alpha-amylase is increased more with an increase in temperature than is beta-amylase. The lowest ratios were

TABLE I

INFLUENCE OF THE TEMPERATURE AND TIME OF MALTING AT 45% AND 48% MOISTURE ON THE AMYLASE CONTENT OF THE MALTS OF THREE BARLEY SAMPLES

Malting temperature	Malting moisture %	Malting time days	Diastatic power	Amylase		Ratio beta/alpha
			Maltose equivalent	Beta-maltose equivalent	Alpha-dextrinizing 20°C unit	
WISCONSIN BARBLESS						
12°C—constant	45	4	384	366	6.8	53.8
	45	6	460	422	12.3	34.3
	45	8	492	444	14.9	29.8
	45	10	544	480	19.0	25.3
	48	4	384	366	6.9	53.0
	48	6	440	405	11.7	34.6
	48	8	516	459	17.2	26.7
	48	10	560	488	21.0	23.2
16°C—constant	45	4	444	391	16.0	24.4
	45	6	496	431	19.3	22.3
	45	8	488	416	21.2	19.6
	45	10	492	420	21.3	19.7
	48	4	472	424	14.8	28.6
	48	6	536	481	18.9	20.4
	48	8	544	478	22.1	21.6
	48	10	580	506	23.6	21.4
20°C—constant	45	4	448	387	18.3	21.1
	45	6	492	431	20.4	21.1
	45	8	516	450	22.1	20.4
	45	10	508	441	22.2	19.9
	48	4	572	508	19.0	26.7
	48	6	576	511	21.6	23.7
	48	8	588	521	22.4	23.3
	48	10	560	491	22.6	21.7
20°C—2 days, dropped to 12°C, and held for the remaining time	45	4	500	446	16.3	27.4
	45	6	604	537	20.0	26.9
	45	8	572	500	21.2	23.6
	45	10	624	537	25.4	21.1
	48	4	540	492	14.9	33.0
	48	6	584	513	23.4	21.9
	48	8	572	496	22.0	22.5
	48	10	648	566	23.8	23.8

TABLE I—*Continued*

Malting temperature	Malting moisture %	Malting time days	Diastatic power	Amylase		Ratio beta/alpha
			Maltose equivalent	Beta-maltose equivalent	Alpha-dextrinizing 20°C unit	
WISCONSIN HYBRID H35-7-2-1-3						
12°C—constant	45	4	680	641	12.2	52.5
	45	6	792	710	24.9	28.5
	45	8	900	790	33.1	23.9
	45	10	904	777	37.8	20.6
	48	4	700	667	11.2	59.6
	48	6	824	743	23.4	31.8
	48	8	904	784	31.0	25.3
	48	10	928	800	38.4	20.8
16°C—constant	45	4	728	646	26.0	24.8
	45	6	796	672	37.1	18.1
	45	8	896	758	40.7	18.6
	45	10	880	722	46.2	15.6
	48	4	804	718	27.4	26.2
	48	6	896	772	37.2	20.8
	48	8	964	812	44.3	18.3
	48	10	952	790	47.2	16.7
20°C—constant	45	4	784	669	34.8	19.2
	45	6	840	710	38.8	18.3
	45	8	828	685	41.8	16.4
	45	10	848	705	42.4	16.6
	48	4	812	702	33.2	21.1
	48	6	868	743	37.3	19.9
	48	8	844	701	42.0	16.7
	48	10	832	684	47.5	14.4
20°C—2 days, dropped to 12°C, and held for the remaining time	45	4	720	645	21.9	29.5
	45	6	820	713	30.2	23.6
	45	8	872	753	36.2	20.8
	45	10	884	746	40.7	18.3
	48	4	684	616	19.9	31.0
	48	6	908	825	26.4	31.3
	48	8	976	844	39.1	21.6
	48	10	904	766	40.9	18.7
PEATLAND						
12°C—constant	45	4	736	679	17.2	39.5
	45	6	844	736	32.8	22.4
	45	8	1032	864	47.8	18.1
	45	10	1112	902	63.7	14.2
	48	4	708	633	17.8	35.6
	48	6	868	745	34.2	21.8
	48	8	1048	890	45.7	19.5
	48	10	1132	927	62.4	14.9

TABLE I—*Continued*

Malting temperature	Malting moisture %	Malting time days	Diastatic power	Amylase		Ratio beta/ alpha
			Maltose equivalent	Beta-maltose equivalent	Alpha-dextrinizing 20°C unit	
PEATLAND						
16°C—constant	45	4	844	710	40.2	17.7
	45	6	968	782	58.2	13.4
	45	8	1072	835	71.8	11.6
	45	10	1168	882	84.6	10.4
	48	4	884	750	40.0	18.8
	48	6	1000	826	55.0	15.0
	48	8	1108	877	70.0	12.5
	48	10	1112	873	72.3	12.1
20°C—constant	45	4	912	740	55.0	13.5
	45	6	976	758	65.6	11.6
	45	8	1020	790	69.3	11.4
	45	10	968	729	72.5	10.1
	48	4	944	773	53.8	14.4
	48	6	1012	793	66.0	12.0
	48	8	1004	767	72.0	10.7
	48	10	1016	763	75.8	10.1
20°C—2 days, dropped to 12°C, and held for the remaining time	45	4	853	752	30.8	24.4
	45	6	976	800	51.2	15.6
	45	8	1100	895	62.3	14.4
	45	10	1116	886	70.0	12.7
	48	4	828	743	27.0	27.5
	48	6	948	776	49.8	15.6
	48	8	1040	832	63.0	13.2
	48	10	1104	857	73.5	11.7

produced at 20° by the Wisconsin hybrid and Peatland Malts, and at 16° in Wisconsin Barbless Malts.

Malts produced from Wisconsin Barbless and Wisconsin hybrid H35-7-2-1-3 show approximately the same proportion of the two components, although the latter is much higher in activity. The Peatland malts are highest in activity and show a lower ratio of beta- to alpha-amylase under all conditions.

Discussion

It should be emphasized that the conditions of malting employed in these studies are in many instances completely unsuitable for commercial operations, and the malts prepared by the use of some of the methods would not be acceptable to the industry. The malting losses, particularly at 20°C, were often above a level considered economically feasible by the maltster. Increasing amylase activity by the methods described herein may be disadvantageous because of the long times

involved at the low temperatures or the high malting losses at the higher temperatures. Therefore, the results of these experiments should not be considered directly applicable to commercial malting, but rather as studies designed to understand more completely the physiology of activation of the amylases accompanying malting.

The differential effects of malting moisture upon the development of the two amylase components are of considerable interest. Both diastatic power and beta-amylase were significantly higher when the barleys were germinated at high (48%) moisture. This moisture effect is in agreement with earlier observations of Shands *et al.* (1941), of Dickson and Burkhart (1942), and of Sallans and Anderson (1939). Alpha-amylase was similar for both moistures used. Dickson and Burkhart (1942) found a significant increase in alpha-amylase activity accompanying higher (48%) malting moisture. It is significant to note that the low moisture level used by Dickson and Burkhart was 43%, or 2% lower than that used in the present study. It would appear from this that moistures below 45% may affect the production of alpha-amylase. At moistures above 45% there is little moisture effect. This observation requires further experimental confirmation using a larger number of barleys.

The length of germination time was an important factor in the development of amylase activity. As might be expected, the time effect was more noticeable at the lower malting temperatures, where growth was slower. Anderson and Sallans (1938) showed that diastatic power increased at 50°F through 13 days of malting but reached a maximum value after 9 days at 56°F. The differential response of samples to germination time with respect to rate of beta-amylase production is in harmony with the observations of Weichherz and Asmus (1931), and Anderson and Meredith (1938). An actual loss of beta-amylase activity at 20°C and high moisture was observed in some of the samples studied. Loss of beta-amylase activity on germination has been observed by Kneen, Miller, and Sandstedt (1942) with wheat.

Alpha-amylase activity increased with time of germination, irrespective of variety or temperature of malting, although Wisconsin Barbless had low activity and was not very responsive. There was also little effect of temperature of malting above 16°C on the final yield of alpha-dextrinizing activity. It would have been interesting to extend germination under these conditions to determine whether or not definite temperature effects would have shown up at later stages in the Wisconsin Barbless Malts.

Higher malting temperatures have for the most part increased initial alpha-amylase activities in the malts, and, as mentioned pre-

viously, decreased beta-amylase content, particularly in the later stages of germination. In the main, the temperature change series malts assumed a position between the 12°C and 16°C series, but resulted in increased yield of beta-amylase in the high moisture Wisconsin hybrid malts at 6 and 8 days germination, and in all the Wisconsin Barbless malts.

It is felt the present study represents an approach to more detailed studies of the physiology of amylase development accompanying the malting of barley under controlled conditions, but the results obtained must be considered as preliminary. The malting moisture effects require further study as indicated. An understanding of variation caused by the barley variety and the environment prevailing during the growing season in relation to the response of the barley to malting would be of value.

Summary

Individual samples of three varieties of barley, Wisconsin Barbless, Wisconsin hybrid H35-7-2-1-3, and Peatland were each malted under different conditions of moisture, temperature, and time. Four malting temperature schedules (12°, 16°, and 20°C constant, and 2 days at 20°C then dropped to 12°C), two malting moistures (45 and 48%), and four germination times (4, 6, 8, and 10 days) were used, giving a total of 32 combinations.

The influence of the various malting conditions on the development of the amylase components is reported. Time of germination was the most important single factor in the development of alpha-amylase activity. Malting temperatures above 16°C resulted in more rapid development of alpha-amylase, but affected the final values only slightly. Alpha-amylase was usually unaffected by malting moisture.

In general, maximum beta-amylase activity was obtained more rapidly as the temperature of malting increased. At 20°C, and occasionally at 16°C, further malting resulted in malts of somewhat lower beta-amylase activity. Beta-amylase values were usually higher in malts germinated at the higher moisture.

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THIAMINE DISTRIBUTION IN WHEAT, RYE, BARLEY, AND OATS

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One of the important objectives of modern milling is to retain increasing amounts of the vitamins and other valuable grain nutrients in the milled products intended for human consumption. In order to succeed in this endeavor, it is necessary to learn more about the distribution of the different nutrients in the kernel. A number of reports concerning the distribution of nutrients in different grains have been published, but these deal largely with the location of thiamine in the wheat kernel. The purpose of this investigation was to obtain further information concerning the distribution of thiamine in rye, barley, and oats as well as wheat and present the data on these different grain types on a comparable basis.

Scheunert and Schieblich (1937), Schultz, Atkin, and Frey (1939), Pulkki and Puutula (1941), Sherwood, Nordgren, and Andrews (1941), Shetlar and Lyman (1941), Jackson, Doherty, and Malone (1943), Abdon and Laurell (1944), Hoffer, Alcock, and Geddes (1944), Jones

and Moran (1946), and others have shown that commercial wheat flours and mill streams differ in thiamine content and that some parts of the wheat kernel contain more thiamine than the others. In the commercial milling process, it is not possible to divide the different tissues into precisely separated streams, since each mill stream contains particles from several parts of the grain. For this reason, analysis of the various mill streams cannot reveal with certainty the vitamin content of the different parts of the kernel, although useful generalizations can be made. The thiamine content of the coarse bran from a commercial mill, for example, is higher than that of the original whole grain on the average, and its disposal as feed would accordingly constitute a loss of thiamine. Pulkki and Puutula (1941) have shown, however, that the outermost layers of bran are considerably lower in thiamine content than the original grain. Somers, Coolidge, and Hamner (1945), by means of a new photographic method, and Pulkki and Puutula (1946) have recently shown that the thiamine content of the surface bran layers of wheat is no more than a quarter of the average content of the whole grain.

It has been shown by Pulkki and Puutula (1941) and Whiteside (1941) that the major part of the thiamine in a wheat kernel is concentrated in the germ end. Hinton (1942, 1944), Ward (1943), and Jackson, Doherty, and Malone (1943) have reported that the wheat tissues richest in thiamine appear to be the scutellum and the tissues adjoining it. Likewise in rye, barley, and oats, the germ portion, especially the scutellum, has been shown to be rich in thiamine by Ihde and Schuette (1941) and Hinton (1944). In the present report, emphasis is given to the relationship between thiamine and fat content, as was pointed out earlier by Pulkki and Puutula (1941 and 1946), as well as by Abdon and Laurell (1944). The relation of thiamine to ash content is also brought out, even though this relationship is generally known. Fiber was determined in most of the samples. Special attention was paid to the bran coats of the different grains since the nutritive value of such material has been difficult to estimate.

Materials and Methods

All the grains used in these experiments were grown in Finland except the wheat, which was imported Manitoba No. 2. The varieties used were rye, Toivo (Jokioinen); barley, Binder (Abed); and oats, Guldregn II (Svalöf). Only normally developed, clean kernels were used. Any foreign material and broken kernels were removed from the samples tested. The samples were not washed, however, to avoid a possible loss of thiamine through leaching. In the case of oats, the kernels were hulled and the groats only were used.

The scouring treatment was accomplished in an apparatus consisting essentially of an emery disc, 525 mm in diameter and 70 mm thick, which revolved in a semicircular trough at 150 RPM. The bottom of the trough was furnished with narrow slits 0.7 × 40 mm in size. The material removed from the grains as well as the stocks remaining were weighed at certain intervals. When kernels were dissected, the cut was made near the germ end in such a manner that the germ, with scutellum included, remained intact but separate from the larger (4/5) part of the kernel.

Thiamine was determined by modifications of the thiochrome method. For wheat and barley, the method described by Pulkki and Puutula (1946) involving treatment of the sample with 0.1 *N* sulfuric acid followed by digestion with diastase and papain was used in preparing the extract. For the rye assays, the filtered digest was prepared in the same manner as for wheat and barley, then purified by extraction with butanol in acid solution before oxidation with potassium ferricyanide. Unless the purification step is employed, Pulkki and Puutula (1946a) have shown that low thiamine values are obtained on rye. The quick potassium chloride extraction method of Andrews and Nordgren (1941) was found suitable for oats. The Pulfrich photometer (filter K7) was used for the fluorescence measurements.

Crude fat was determined by extracting the dried samples with ethyl ether in the Twisselmann apparatus.

Results and Discussion

The ash, thiamine, fat, and fiber contents of the material obtained during the progressive scouring of wheat are presented in Table I. It is apparent that the inner portion of the wheat kernel contains comparatively little thiamine. About 80% of the total wheat thiamine was contained in the material obtained by scouring off the outer 50% of the kernels. The first of the material removed from wheat by scouring consists almost entirely of the outer layers of the bran (pericarp). As shown in Table I, the thiamine content of this material when representing less than 0.5% of the wheat weight may be as low as 0.9 μg/g, which is only one-fourth of that in the whole grain. The fiber content, however, is very high. Bran from a commercial mill, of course, contains many times this amount of thiamine. As the scouring progresses past the outer bran layers, portions of the embryo begin to be removed, as may be seen in the sharp rise in both fat and thiamine. It is to be noted that the thiamine reached a maximum when the first 25% of the grain had been scoured away. When the kernels are scoured in this manner, considerable endosperm is removed before the scutellum is reached. Thus, the results are in fair agree-

TABLE I

THIAMINE, ASH, FAT, AND FIBER CONTENT OF MATERIAL REMOVED IN THE PROGRESSIVE SCOURING OF WHEAT KERNELS
(ANALYTICAL DATA EXPRESSED ON DRY MATTER BASIS)

Material removed	Thiamine μg/g	Ash	Crude fat	Crude fiber
% %	%	%	%	%
0.0 – 0.42[1]	0.9	3.06	1.82	25.2
0.42– 1.2	1.2	2.84	2.14	24.7
1.2 – 1.9	1.6	3.03	3.19	25.4
1.9 – 3.1	3.3	3.93	5.26	19.9
3.1 – 4.3	4.3	4.89	6.67	15.5
4.3 – 5.4	5.4	5.15	6.80	8.7
5.4 – 7.1	6.3	5.05	6.45	10.9
7.1 – 9.1	5.9	4.65	6.04	—
9.1 – 10.9	6.3	4.31	5.55	7.7
10.9 – 14.6	6.8	3.84	5.07	—
14.6 – 17.3	7.1	3.48	4.62	—
17.3 – 20.1	8.2	3.18	4.43	—
20.1 – 25.0	8.3	2.77	3.74	4.1
25.0 – 30.0	6.7	2.40	3.52	—
30.0 – 35.0	5.7	2.04	2.94	—
35.0 – 40.4	5.2	1.77	2.49	—
40.4 – 45.6	4.0	1.56	2.28	—
45.6 – 50.0	3.2	1.48	2.05	—
50.0 –100	1.5	0.88	1.09	0.6
0 –100[2]	3.8	1.81	2.18	3.1

[1] With reference to the seeming overlapping of fractions, the authors advise that the values given in the right-hand column represent the highest percentage of material included in the fraction indicated. For example, the first fraction contains up to and including 2.0% of the sample, whereas the second fraction contains the material remaining in excess of 2.0% and up to and including 4.3%. *Editor.*

[2] Original wheat.

ment with those of Hinton (1944), who reported that most of the thiamine in wheat is concentrated in the scutellum and its immediate vicinity.

Table II gives the results of corresponding experiments with rye. They are quite similar to those obtained with wheat, as would be expected in view of the similarity in kernel structure of wheat and rye. As with wheat, it is possible to remove 3 to 4% of the outer rye kernel by scouring and find that the thiamine content of this material is lower than the average content for the whole grain. As the scouring progressed, the fat content reached a maximum somewhat before the thiamine. This relationship was not as definite for rye as it was for wheat. Even though the scouring may remove the different tissues of rye in slightly different order than in wheat because of the differences in kernel shape, it is clear from these results that the major portion of the thiamine in rye is concentrated in the scutellum and the adjacent tissues. This was confirmed by measuring the thiamine content of the germ end and the remaining part of rye kernels, as well as those from hulled oats and barley. The kernels were dissected in such manner

TABLE II

THIAMINE, ASH, FAT, AND FIBER CONTENT OF MATERIAL REMOVED IN THE PROGRESSIVE SCOURING OF RYE KERNELS
(ANALYTICAL DATA EXPRESSED ON DRY MATTER BASIS)

Material removed	Thiamine µg/g	Ash	Crude fat	Crude fiber
% %	%	%	%	%
0.0– 2.0[1]	1.7	4.14	2.82	13.8
2.0– 4.3	2.9	4.10	5.10	12.8
4.3– 7.2	5.0	5.22	6.77	11.0
7.2– 10.0	5.4	5.70	6.74	6.1
10.0– 15.4	5.8	5.00	4.96	—
15.4– 21.4	5.4	3.90	3.83	1.6
21.4– 30.1	5.4	2.98	3.00	—
30.1– 39.7	4.2	2.33	2.36	1.4
39.7– 50.0	3.3	1.85	2.07	1.1
50.0–100.0	1.1	1.02	0.89	1.0
0–100[2]	2.8	2.10	1.81	3.0

[1] See footnote 1, Table I.
[2] Original rye.

that only about 20% of the kernel weight was left on the germ end. The results are presented in Table V and show that, in the case of rye, at least 70% of the thiamine occurs in this part of the kernel.

The results of similar scouring experiments on barley are given in Table III. Even though barley differs from wheat and rye in being

TABLE III

THIAMINE, ASH, FAT, AND FIBER CONTENT OF MATERIAL REMOVED IN THE PROGRESSIVE SCOURING OF BARLEY KERNELS
(ANALYTICAL DATA EXPRESSED ON DRY MATTER BASIS)

Material removed	Thiamine µg/g	Ash	Crude fat	Crude fiber
% %	%	%	%	%
0.0 – 2.35	1.3	11.44	1.98	24.2
2.35– 4.85	1.5	8.37	1.73	31.1
4.85– 10.35	4.4	7.28	4.42	27.1
10.35– 15.20	9.2	7.60	8.15	15.8
15.2 – 20.20	9 8	6.78	8.30	6.0
20.2 – 30.00	6.8	4.50	5.66	2.2
30.0 –100.00	2.0	1.02	1.34	1.1
0 –100[2]	3.4	2.70	2.00	5.2

[1] See footnote 1, Table I.
[2] Original barley.

more chaffy, it may be seen that the distributions of thiamine and fat in barley are very similar to those for wheat and rye. The greatest difference occurs in the distribution of ash. The outer protective tissues of the barley kernel were found to be comparatively poor in thiamine. As the scouring was continued, a maximum thiamine

content was obtained when about 20% of the kernel was removed. This is somewhat earlier than one would expect in view of the much thicker protecting layers in barley than in wheat and rye. In the dissection experiments, the germ end of the barley kernel was observed to contain about two-thirds of the total thiamine (Table V).

TABLE IV

THIAMINE, ASH, AND FAT CONTENT OF MATERIAL REMOVED IN THE PROGRESSIVE SCOURING OF HULLED OAT KERNELS
(ANALYTICAL DATA EXPRESSED ON DRY MATTER BASIS)

Material removed	Thiamine μg/g	Ash	Crude fat
% %	%	%	%
0.0– 1.0	14.4	5.11	10.1
1.0– 3.2	14.4	5.20	10.4
3.2– 6.3	14.4	4.90	11.9
6.3– 10.3	13.2	4.27	11.4
10.3– 14.7	9.3	3.70	10.6
14.7– 19.8	9.7	3.96	9.85
19.8– 29.9	6.5	2.88	8.73
29.9– 40.1	5.7	2.22	7.62
40.1– 49.8	4.4	2.03	7.00
49.8–100.0	3.5	1.40	5.65
0 –100[2]	5.6	2.12	6.65

[1] See footnote 1, Table I.
[2] Original oats (hull removed).

Table IV presents the results obtained in similar experiments with oats. It is readily apparent from these data that the distribution of thiamine in hulled oats is considerably different from that in wheat or rye. The outer surface layers of the oat kernel (pericarp) were observed to contain considerably more thiamine than the whole kernel. Such material from oats contained 10 to 15 times as much thiamine as corresponding material scoured off from wheat and rye. Although the hulled oat kernel appears to be similar to wheat and rye kernels, it differs in structure, mainly in that the pericarp of oats is thinner and is of more simple construction than that of wheat and rye (Winton and Winton, 1932). The highest thiamine content in the oat scourings was 14.4 μg/g, representing mainly pericarp tissue. In view of Hinton's (1944) observations, it is probable that even higher concentrations could be found in the scutellum, although it was not possible to demonstrate this by the method of experimentation used in this study. A weak maximum occurred in the thiamine concentration when the material removed by scouring amounted to 15 to 20% of the kernel weight.

The dissection results in Table V indicate that there is less difference in germ and endosperm thiamine content in oats than in wheat, barley,

TABLE V

GRAIN DISSECTION EXPERIMENTS. THIAMINE, ASH, AND CRUDE FAT CONTENTS ON THE ENTIRE GRAIN, GERM ENDS, AND MAIN PARTS OF WHEAT, RYE, BARLEY, AND HULLED OAT KERNELS

(ANALYTICAL DATA EXPRESSED ON DRY MATTER BASIS)

Determination	Wheat	Rye	Barley	Hulled oats
ENTIRE KERNEL				
Thiamine, % of total	4.4	3.2	4.7	6.6
Ash, %	1.77	2.08	2.60	2.12
Fat, %	1.9	1.7	1.7	7.7
GERM END				
Weight, % of whole grain	18.0	19.7	22.7	19.4
Thiamine—				
μg/g	18.9	11.4	13.8	13.7
% of total	77.6	69.8	67.0	40.3
Ash, %	2.72	3.0	3.52	2.62
Fat, %	5.7	4.8	4.3	11.1
MAIN PART (KERNEL WITH GERM END REMOVED)				
Weight, % of whole grain	82.0	80.3	77.3	80.6
Thiamine—				
μg/g	1.2	1.2	2.0	4.9
% of total	22.4	30.2	33.0	59.7
Ash, %	1.56	1.86	2.32	1.99
Fat, %	1.1	0.9	1.0	6.9

or rye. In oats, about 60% of the thiamine is located outside the germ area. The thiamine appears to be more evenly distributed throughout the kernel in oats than in the other cereals. Thus, products made from oat endosperm would be comparatively good sources of thiamine, even though the vitamin-rich, outer surfaces are removed by polishing.

Samples of oat products obtained from a large commercial mill were assayed for thiamine with the following results:

Product	Thiamine, μg/g (dry basis)
Whole oats	4.0
Hulls	0.9
Kernels before polishing	5.0
Polished kernels	4.9
Polishing dust	10.7
Steamed and rolled oats	4.1

These data show that in the commercial polishing process, material very rich in thiamine is removed from the oats. In these samples, the difference in the thiamine content between the polished and unpolished

kernels was insignificant, due to the very small amount of material removed.

Oat hulls separated by hand were found to contain from 0.1 to 0.4 μg/g thiamine. Oat hulls from a commercial mill always contain some material from the kernel and consequently contain more thiamine than the pure hulls separated by hand. The thiamine content of the hulls, however, is too low to be of practical importance.

These results show that there is a remarkably close relationship between thiamine content and fat content, in many cases closer than that between thiamine and ash. This is particularly striking in the case of barley but holds for the other cereals as well. In oat bran a high thiamine content is accompanied by a high fat content, whereas the pericarp of wheat and rye is low in both thiamine and fat. Abdon and Laurell (1944) have reported that the fat and vitamin content of each series of mill products of a commercial wheat mill are closely related. The experimental data presented by Jones and Moran (1946) confirm this observation. When commercial flours are blended to definite ash levels, the thiamine, ash, and fat contents generally follow each other in a regular pattern. Thus, a cereal product having a high natural fat content may be expected to contain a relatively high level of thiamine.

Scouring is generally used commercially in the preparation of barley products. These experiments reveal that in a heavy scouring treatment substantial amounts of thiamine may be lost in some cases. Should scouring be applied to wheat and rye, however, products containing significantly less fibrous material than whole meal could be prepared which would retain nearly all of the natural thiamine. Further investigations are needed to reveal the amounts of other vitamins and important nutrients in the bran layers of different cereals.

Summary

The distribution of thiamine in wheat, rye, barley, and hulled oat kernels was compared by assaying the material successively removed from these grains by means of an emery scouring machine.

The major portion of the thiamine occurs in or near the germ in rye, barley, and wheat kernels. In oat kernels the thiamine is more evenly distributed throughout the kernel.

The outer bran layers (pericarp) of wheat and rye contained only small amounts of thiamine, whereas the corresponding tissues of the oat kernel were very rich in thiamine. The hulls of oats contained only small quantities of thiamine.

Dissection experiments confirmed the conclusions drawn from the scouring studies.

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EFFECT OF POLYOXYETHYLENE STEARATE ON THE CRUMB SOFTNESS OF BREAD

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The importance of bread staling and a review of the large amount of literature devoted to it has been presented by Cathcart (1940) and more recently by Geddes and Bice (1946). Revised concepts of the structure and composition of starch and the mechanism of bread staling have been reviewed by Noznick, Merritt, and Geddes (1946) and Schoch (1947). The separation of starch into straight and branched chain components shows promise of throwing considerable light on the problem. From the consumer standpoint, however, bread still becomes stale when it loses its softness, fresh flavor, and eating quality, and the baker is continually searching for some means of retarding the loss of any or all of these qualities. The discovery of a material which has a pronounced retarding effect on the rate at which bread crumb loses its softness with age is reported here.

Food emulsifier investigations, which included work on hexitol anhydride esterification products and their polyoxyethylene derivatives, led to the discovery that polyoxyethylene stearate had a remarkable softening effect on starch gels and the ability to retard the stiffening of bread crumb with age. It seemed to have no other effects on the production or character of bread and would therefore be particularly valuable to the baker.

Polyoxyethylene stearate, as referred to here, is the reaction product of ethylene oxide and stearic acid containing an average of eight oxyethylene groups per stearic acid radicle. This material is a light-colored paste, hard at 20°C or below, and melting at 30°C. It is readily dispersed in flour-water doughs mixed by conventional methods, and equally effective in solid or liquid form. Tests so far have shown it is nontoxic,[1] hydrolyzed by pancreatic lipase *in vitro*, stable to heat and aging, and imparts no flavor or odor to products in which it is used.

Effect of Polyoxyethylene Stearate on Dough and Bread Properties

Baking Formula and Procedure. Preliminary experiments indicated that the effect of this material could be demonstrated with starch pastes and straight dough or sponge-dough process bread, but to make

[1] Results of toxicity studies made on this material are available on request from the R. T. Vanderbilt Co., Inc.

the results as practical as possible the following formula and procedure yielding six one-pound loaves was used.

	Sponge g	Dough g	Total flour basis
Flour (Northwestern Patent)	1080.	720.	100.00
Water	650.	500.	64.00
Yeast	54.	—	3.00
Yeast food	5.	—	.28
Sugar	—	90.	5.00
Salt	—	39.	2.20
Shortening	—	18.	1.00
Milk powder	—	72.	4.00

Sponges were fermented three and a half hours at 30°C and remixed with dough ingredients in a 15 quart Read mixer, equipped with a single hook, to the point shown in the farinograms of Figure 2. Doughs were divided from the mixer, given 30 minutes rest, made up in a National sheeter and moulder, then panned and proofed to height at 30°C. The loaves were baked for 30 minutes at 210°C, cooled for one hour in circulating air held at 15°C and 85% relative humidity, wrapped in moisture-proof cellophane and stored on open racks in the laboratory.

The following morning loaf volumes were obtained by seed displacement; one loaf was scored, and the others were rewrapped for aging and compressibility tests.

Effect on Crumb Softness. The A.A.C.C. method for determining the staleness of bread with the Baker compressimeter (*Cereal Laboratory Methods*, 4th ed., 1941) was used with the following modifications.

1. The square plunger face provided with the instrument was used, and a section of crumb of the same size was cut from the slice one inch away from the bottom crust and the side without the "break." This location of the sample was selected to avoid the holes and hard spots often found in the center of a slice.

2. A uniform strain of 2.5 mm was used throughout and readings of the stress necessary to produce it recorded as firmness. The stress in grams read from the scale on the instrument was multiplied by 10 to show actual stress applied by the lever system to the section of bread crumb, and to secure better correlation with similar readings taken from other types of compressibility testers (Platt and Powers, 1940; Combs, 1944).

The effects of adding 0.5, 1.0, and 2.0% (flour basis) polyoxyethylene stearate on the firmness of crumb and its rate of change with age as determined by the above method are shown in Table I and Figure 1. The ester was added at the dough stage or "remix" and no other change in formula or procedure was made. Values shown are averages from four separate baking tests.

TABLE I

EFFECT OF INCREASING AMOUNTS OF POLYOXYETHYLENE STEARATE ON FIRMNESS OF BREAD CRUMB

Ester	Firmness as stress per 2.5 mm strain		Slope [1]
	1-day-old	3-days-old	
%	g	g	
0	90	170	1.000
0.5	71	125	.675
1.0	64	89	.316
2.0	66	88	.280

[1] Slopes of lines joining the one- and three-day firmness readings.

The difference in firmness of the control loaf and that containing 0.5% ester at one day was readily discernible by squeezing the loaf, but that between the loaves containing various amounts of ester was not. The control loaf at three days felt firm or stale, while those containing 0.5% and 1.0% ester felt progressively softer, and the 1% and 2% ester loaves could not be distinguished from one-day-old controls by this squeeze test. The slopes of lines joining the one- and three-day firmness readings show that the rate of development of firmness of crumb or staleness has been progressively reduced as the quantity of ester was increased. This difference in softness and an increased tenderness of crust and crumb were the only characteristics showing any change whatever when the bread was scored at one day of age, so bread scores are not shown. The fact there was no change in external or internal appearance of the loaves containing the ester was of great importance, however, for it indicated that there had been no significant effect on the condition of the gluten or the rate and extent of fermentation of the dough.

Although the baking tests failed to show any effect of this material on breadmaking, a more detailed examination of each step in the process was carried out in the hope of finding some clue to the mechanism of its action.

Effect on Absorption and Mixing. At normal dough temperatures (20° to 30°C) the ester is a soft paste and is readily dispersed through a dough without pretreatment or special mixing. Farinograph curves of 480 g charges of flour-water doughs and also of doughs containing all the ingredients, with various amounts of the ester (without adjustment of absorption), are shown in Figure 2. The percentage of ester was based on the weight of flour, and the "complete-formula" doughs were taken from the "remix" of the baking tests reported above.

These curves show that polyoxyethylene stearate retards the rate of dough development but does not increase the time to maximum

development or the breakdown with overmixing. It does reduce consistency about 20 Brabender Units for each 0.5% added, but this corresponds to a change in absorption of about 0.7% which is not enough to affect bread quality.

The changes in rate of development and consistency noted are similar to those obtained when shortening is added to a dough (Moore

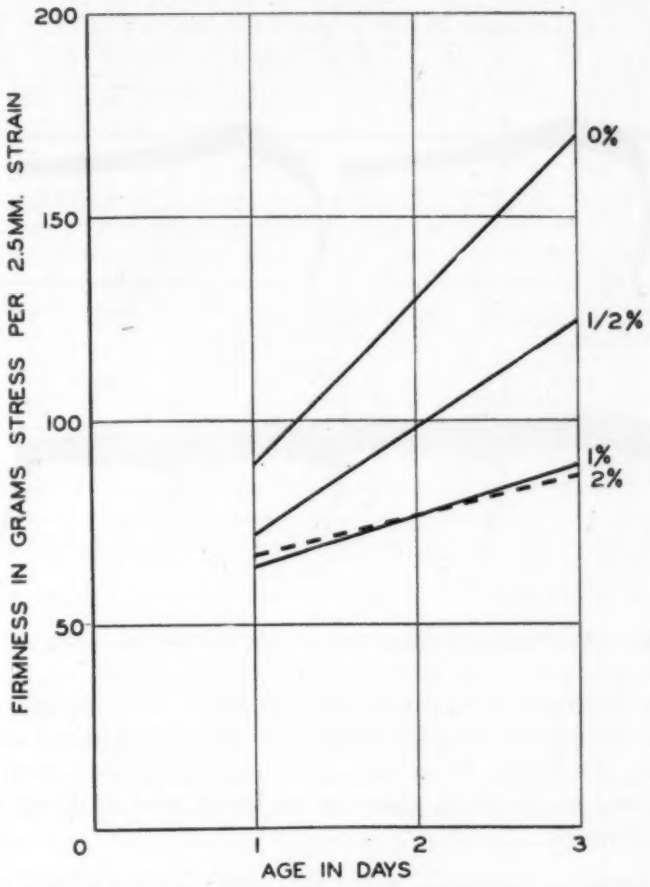

Fig. 1. Effect of increasing amounts of polyoxyethylene stearate on firmness of bread crumb.

and Herman, 1942), and at concentrations of ester normally used (0.25 to 0.5%) are not great enough to require adjustment in mixing time or absorption.

Effect on Fermentation and Handling. The possible effects of the ester on doughs during fermentation were investigated by conducting gas production tests by the pressuremeter method (*Cereal Laboratory Methods*, 4th Ed., 1941) on sponges and doughs, and by observation of

"feel," ease of make-up, and proofing time during the baking tests. Differences in the amount of gas produced in the pressuremeter between sponges or doughs with and without 1% (flour basis) polyoxyethylene stearate were well within the experimental error of the method and showed that this material had no effect on the activity of the yeast.

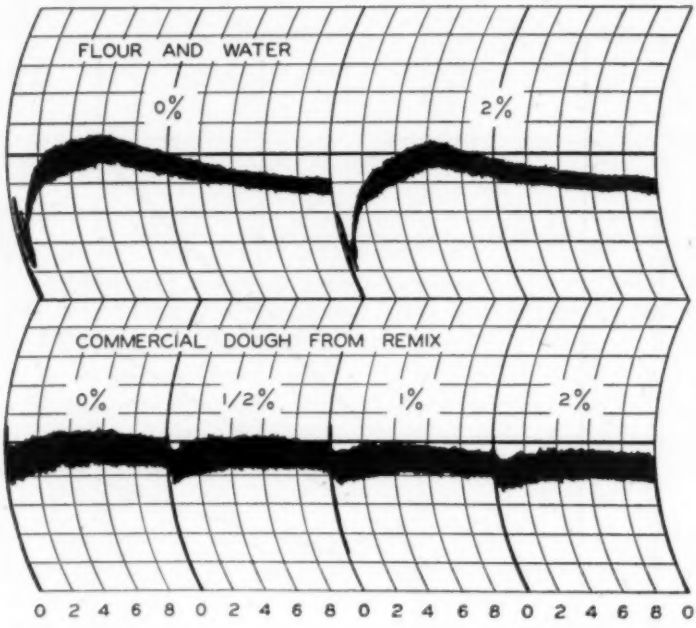

Fig. 2. Effect of polyoxyethylene stearate on mixing characteristics and consistency of doughs.

Three experienced operators were unable to find any difference in handling or make-up properties between the control dough and those containing the ester. Proof times (to a predetermined height) were identical and oven spring, character of break, and final loaf volumes were unchanged.

Effect of Polyoxyethylene Stearate on Viscosity of Starch Pastes

Since the softening effect on the bread crumb was not accompanied by any measurable effect on the condition of the gluten or the structure of the loaf, it was probable that it was due to a change in the starch. The amylograph has proved to be valuable for studying the properties of starch pastes, as shown by Anker and Geddes (1944) and Brown and Harrel (1944), and could be adapted to studies on gelatinized starch if the concentrations of starch were kept low enough to produce soft gels after standing.

The first tests were made on 6% suspensions of various kinds of starch with and without 2.0% (dry basis) polyoxyethylene stearate added to the suspension before heating. Four hundred grams of each suspension were heated in the amylograph at the rate of 1.5°C per minute from 40°C until maximum viscosity was obtained and transferred to glass jars as soon as the viscosity started to decrease. The starch pastes were stored overnight at room temperature and then 300 g returned to the machine and mixed for 10 minutes at 30°C. The maximum hot viscosity and the minimum viscosity of these pastes the next day (multiplied by the factor 1.33 to correct for the smaller charge) are shown in Table II.

TABLE II

EFFECT OF POLYOXYETHYLENE STEARATE ON THE VISCOSITY OF VARIOUS KINDS OF STARCH PASTE

Starch	Maximum hot viscosity			Minimum cold viscosity, next day [1]		
	Control	+2% Ester	Change	Control	+2% Ester	Change
	BU [2]	*BU*	%	*BU*	*BU*	%
Wheat	50	70	+40	466	207	−56
Corn	170	220	+30	1080	500	−54
Tapioca	370	360	− 3	760	427	−43
Waxy corn	600	485	−19	587	507	−14
Potato	610	440	−28	1333	563	−58

[1] Minimum viscosities were computed from the actual values by multiplying by the factor 1.33 to compensate for the smaller charge used as compared with that taken in determining maximum hot viscosities.
[2] Brabender units.

The ester increased the hot paste viscosities of wheat and corn starch, had no effect on tapioca starch, and decreased that of waxy corn and potato starch. All viscosity readings taken after 10 minutes' mixing the next day and corrected to 400 g show an increase over the maximum when made, except for the waxy corn control. However, on the next day the pastes containing the ester show a significant decrease in viscosity when compared to the controls of the same age. This decrease is not great for the waxy corn, but it is around a 50% reduction for the others, which is certainly significant and of the same order for the four kinds of starch irrespective of their comparative gel strength or viscosity. These tests therefore indicated that it was this ability of polyoxyethylene stearate to reduce the viscosity or "set-up" of starch pastes after standing, that was responsible for its effect on bread crumb.

The effect of smaller amounts of ester on starch paste viscosity was shown by preparing a series of 6% corn starch pastes containing 0, 0.5, 1.0, and 2.0% of the ester and treating them as described above.

TABLE III

EFFECT OF CONCENTRATION OF POLYOXYETHYLENE STEARATE ON VISCOSITY OF 6% CORN STARCH PASTE

Ester [1]	Temperature at max. visc.	Viscosity in Brabender units		Reduction in viscosity
		Maximum-hot	Minimum-cold (next day) [2]	
%	°C	*BU*	*BU*	%
0	88.0	170	1080	—
0.5	92.5	210	665	38
1.0	93.0	230	558	48
2.0	93.0	220	500	54

[1] Concentration is expressed as % of dry starch.
[2] Values in Figure 3 ×1.33.

Since the apparent temperature of gelatinization increased with increasing amounts of ester, these and the viscosity data are shown in Table III and the curves themselves in Figure 3.

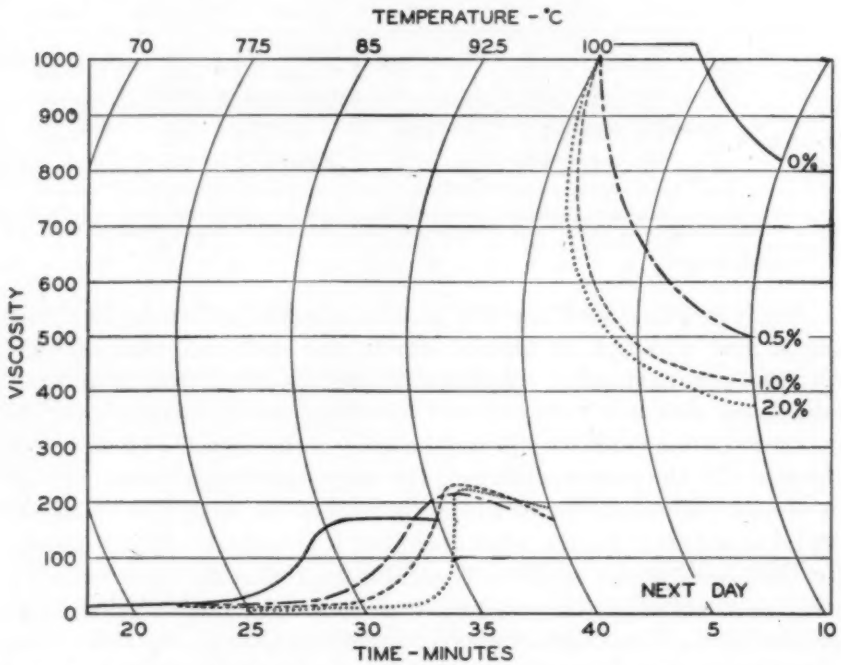

Fig. 3. Effect of concentration of polyoxyethylene stearate on viscosity of 6% corn starch paste.

The increase in apparent temperature of gelatinization is similar to that obtained when the concentration of starch is reduced (Anker and Geddes, 1944) and may well be due to a wetting or lubricating action of the ester. Maximum viscosities increase somewhat as the

concentration of ester increases but do not appear to be particularly significant. It is the progressive decrease in the viscosity of the pastes when remixed the next day that is outstanding and proves that this decrease is truly a function of the additive.

In order to determine whether this material actually affected the progressive setting up or retrogradation of starch paste with further aging, a larger batch of starch was required so that aliquots of paste which had not been disturbed since they were cooked might be measured in the amylograph. Two kilogram batches of 6% corn starch with 0.0, 0.5, and 1.0% (dry basis) polyoxyethylene stearate added were cooked in a 5-quart Merryway cake mixing bowl surrounded by

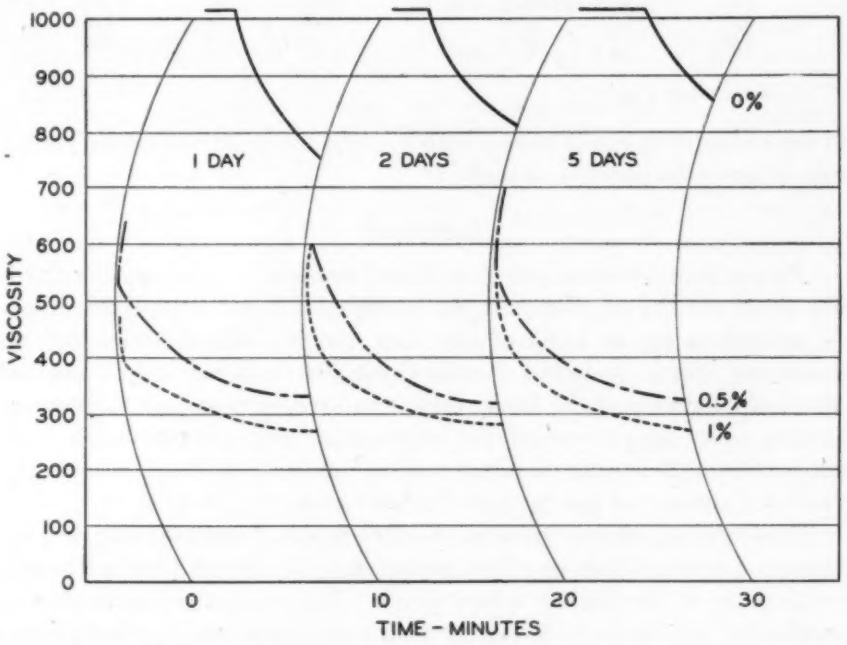

Fig. 4. Effect of polyoxyethylene stearate on the change in viscosity of 6% corn starch pastes with age.

a boiling water bath and with the paddle turning at low speed during the entire cook. Heating for one hour produced a paste temperature of only 90°C, so this paste was not a duplicate of those made in the amylograph and an effect of incomplete gelatinization would be expected in the pastes containing the ester. However, conditions between batches could be held constant and the viscosities obtained were of normal magnitude. To prevent spoilage, 2 ml of formaldehyde solution were added just before each batch was packed in sealed pint jars, and these were then cooled and stored at room temperature.

After one, two, and five days' storage a 300 g charge from each batch was transferred to the amylograph, warmed to 30°C, and run for 10 minutes. The curves obtained and the data taken from them are shown in Figure 4 and Table IV. These show the progressive increase

TABLE IV

EFFECT OF POLYOXYETHYLENE STEARATE ON THE CHANGE IN VISCOSITY OF 6% CORN STARCH PASTES WITH AGE

Ester	Minimum cold viscosity after 10 minutes mixing [1]		
	1-day-old	2-days-old	5-days-old
%	*BU*	*BU*	*BU*
0.0	1020	1065	1150
0.5	445	440	440
1.0	372	378	368

[1] Values in Figure 4 ×1.33.

in the viscosity of starch pastes with age and the ability of polyoxyethylene stearate to prevent or mask it.

Summary

Polyoxyethylene stearate was found to have a pronounced retarding effect on the development of firmness of bread crumb with age. In quantities up to 2.0% of the flour weight, this material had no significant effect on bread doughs during their processing or on the physical character of the bread other than its softness and tenderness. Baking tests supplemented by farinograph and pressuremeter tests showed that there was no effect on the condition of the gluten or the rate and amount of gas produced by the yeast.

Addition of polyoxyethylene stearate to starch pastes increased the apparent temperature of gelation but did not prevent complete gelatinization at the higher temperature. Pastes containing it showed much less "set-back" or stiffening after cooling and storage for one day and no tendency to increase in viscosity or retrograde with more extended aging.

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NIACIN AND PANTOTHENIC ACID CONTENT OF CORN HYBRIDS [1]

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Corn, which is called by some the imperial agricultural plant of America, is of great nutritional importance. From the standpoint of total production, it occupies second place among the food plants of the world. It is the chief grain used in feeding livestock. It is also the chief article of diet of peoples in certain sections of the world, and in such sections the human nutritional disease, pellagra, is quite prevalent.

In recent years the above facts have been recognized, and it is now agreed that some emphasis in corn production should be placed on nutritive value as well as on the previously stressed characters which contribute to a high yield of sound corn on standing stalks. Any information concerning factors that may influence the value of corn as a source of the nutrients, protein, oil, and especially vitamins, would be of inestimable value.

Burkholder, McVeigh, and Moyer (1944) studied the niacin content of 94 samples of yellow corn and found a variation of 11.3 to 36.3 μg of niacin per gram. The locality in which the corn was grown did not appear to have any influence on its niacin content, but a genetic factor may be responsible for the ability of the corn to store niacin. Teply, Strong, and Elvehjem (1942a) noted that environment influenced the niacin, pantothenic acid, and pyridoxine content of wheat. In another study (1942) they reported that the niacin content of corn varied from 1.56 to 2.60 mg %. Ellis and Madsen (1943), in summarizing the available information on the vitamin content of yellow corn, reported that the niacin content of 27 samples was 6.4 mg per pound, with a

[1] Published with the approval of the Director of the Ohio Agricultural Experiment Station.

range of 3.2 to 9.5 mg. The pantothenic acid content of nine samples of yellow corn varied from 2.72 to 4.54 mg per pound, with an average of 3.36 mg. Knox, Heller, and Sieglinger (1944) reported that sorghum grains varied little in riboflavin and pantothenic acid content, while niacin varied as much as 100%, depending on the place where the sorghum was grown.

It is evident that factors are involved which cause these variations in the niacin and pantothenic acid content of grains. These factors may be environmental, such as rainfall, soil, and length of growing season, and/or hereditary. The data reported in this paper show the niacin and pantothenic acid content of various corn hybrids.

Materials and Methods

Nine well-known, double-cross hybrids were selected for analysis for the two vitamins. The hybrids were grown at five Middle West experiment stations over a period of three crop years.

An attempt was made to learn whether variations in vitamin content occurred among the hybrids and, if so, whether such variations could be attributed to hybrid, season, location, or the interaction of any two of these factors.

Preparation of the corn for analysis was as follows: Each sample was finely ground in a Wiley Mill and stored in a tightly closed glass jar in a dark room until analyzed. For niacin assays, an extract was prepared by adding 25 ml 1 *N* sodium hydroxide solution to 1 g of ground corn, autoclaving for 15 minutes at 15 pounds pressure, adjusting the pH to 6.6 to 6.8 with 1 *N* hydrochloric acid solution, and finally diluting the whole to 200 ml. For pantothenic acid assays, the extract was prepared by suspending 1 g ground corn in 75 ml distilled water, autoclaving for 15 minutes at 15 pounds pressure, diluting to 200 ml, and finally filtering with the aid of a thin layer of Celite 585 (Teply *et al.*, 1942a).

Niacin and pantothenic acid were determined microbiologically, using *Lactobacillus arabinosus* as the test organism. The basal medium was that of Krehl, Strong, and Elvehjem (1943). The medium, containing all the ingredients except the vitamins, was prepared at a concentration double that given by Krehl *et al.*, and was kept in the refrigerator under toluene until used. Immediately before each determination the vitamins (also kept in the refrigerator under toluene) were added to the required amount of the concentrated medium, the pH adjusted to 6.6 to 6.8, and the volume diluted to the recommended concentration of Krehl, *et al.*

The test organism was carried on liver tryptone agar stabs. For the inoculum, a 24-hour culture grown in basal medium with added

vitamin and distilled water was treated according to the method of Landy and Dicken (1942).

Triplicate tubes containing 0.05, 0.10, 0.15, 0.20, 0.40, 0.60, and 1.00 μg niacin were used for the standard curve. For pantothenic acid, the amounts used were 0.01, 0.02, 0.04, 0.08, 0.12, 0.16, and 0.32 μg. Duplicate tubes at three levels were used for each corn sample. Aliquots of the extract were of such size that the titrations at each of the three levels fell on the part of the curve which was most nearly linear. Incubation at 31°C for 72 hours was followed by titration with 0.1 *N* NaOH, using brom thymol blue as the indicator. With each determination a reference sample was assayed. The determina-

TABLE I

NIACIN AND PANTOTHENIC ACID CONTENT OF DOUBLE-CROSS HYBRID CORN; 10% MOISTURE
(MILLIGRAMS PER 100 GRAMS)

	1943		1944		1945	
	Niacin	Pantothenic acid	Niacin	Pantothenic acid	Niacin	Pantothenic acid
Hybrid: Ohio C38						
Wooster, Ohio	2.15	0.54	2.08	0.36	1.92	0.47
Lafayette, Ind.	1.91	.51	2.43	.49	2.28	.64
Urbana, Ill.	2.20	.60			1.95	.52
Ames, Iowa	1.98	.69	2.28	.56	1.91	.60
Lincoln, Neb.	2.06	.61	2.50	.43		
Hybrid: Illinois 201						
Wooster, Ohio	2.00	.55	1.85	.36	1.85	.55
Lafayette, Ind.	2.06	.67	2.04	.38	2.35	.61
Urbana, Ill.	2.15	.56	2.01	.40	2.10	.95
Ames, Iowa	1.85	.72	2.27	.32	2.20	.64
Lincoln, Neb.	1.89	.78	1.65	.35		
Hybrid: Illinois 784						
Wooster, Ohio	1.63	.51	1.63	.31	1.43	.73
Lafayette, Ind.	1.87	.40	1.60	.29	1.80	.75
Urbana, Ill.	2.04	.44	1.65	.38	1.50	.61
Ames, Iowa	1.74	.48	1.83	.45	1.62	.79
Lincoln, Neb.	1.72	.64	1.58	.40		
Hybrid: Iowa 939						
Wooster, Ohio	2.46	.64	2.39	.45	2.59	.88
Lafayette, Ind.	2.27	.70	2.65	.48	2.95	.86
Urbana, Ill.	2.31	.70	2.72	.60	2.36	.67
Ames, Iowa	2.32	.51	2.77	.47	2.68	.78
Lincoln, Neb.	2.44	.81	2.58	.37		
Hybrid: U. S. 13						
Wooster, Ohio	1.87	.50	1.98	.21	1.78	.50
Lafayette, Ind.	2.06	.46	1.94	.35	1.94	.54
Urbana, Ill.	2.37	.77	2.32	.39	1.65	.42
Ames, Iowa	2.00	.51	2.23	.48	1.88	.64
Lincoln, Neb.	1.69	.74	1.72	.33		

TABLE I—*Continued*

	1943		1944		1945	
	Niacin	Pantothenic acid	Niacin	Pantothenic acid	Niacin	Pantothenic acid
Hybrid: U. S. 35						
Wooster, Ohio	2.22	0.62	1.90	0.50	2.22	0.73
Lafayette, Ind.	2.27	.53	2.03	.48	2.45	.54
Urbana, Ill.	2.22	.47	2.18	.49	2.12	.44
Ames, Iowa	2.18	.55	1.88	.45	2.05	.60
Lincoln, Neb.	1.99	.68	1.86	.38	—	—
Hybrid: U. S. 44						
Wooster, Ohio	2.07	.50	1.92	.31	2.15	.53
Lafayette, Ind.	2.11	.42	2.36	.36	2.40	.48
Urbana, Ill.	2.24	.52	2.05	.39	2.17	.50
Ames, Iowa	2.14	.50	2.23	.54	2.25	.58
Lincoln, Neb.	2.00	.67	1.77	.35	—	—
Hybrid: Indiana 608C						
Wooster, Ohio	2.21	.43	2.25	.36	2.30	.53
Lafayette, Ind.	2.06	.42	2.59	.39	2.49	.56
Urbana, Ill.	2.23	.50	2.40	.51	2.31	.57
Ames, Iowa	2.17	.49	2.39	.60	2.49	.69
Lincoln, Neb.	2.04	.58	2.19	.47	—	—
Hybrid: Indiana 844D						
Wooster, Ohio	2.14	.44	1.92	.47	2.12	.56
Lafayette, Ind.	2.25	.40	2.44	.40	2.46	.39
Urbana, Ill.	2.30	.58	2.55	.44	2.32	.45
Ames, Iowa	2.19	.41	2.52	.64	2.63	.57
Lincoln, Neb.	2.18	.83	1.97	.50	—	—

tion was accepted if the reference sample gave a value within 10% of its known niacin or pantothenic acid content.

Results and Discussion

The identity of the corn samples and the niacin and pantothenic acid content are shown in Table I. Variations in the content of both vitamins were found in all hybrids, for all three years and at all locations. For the whole study, the niacin values ranged from 1.43 to 2.95 mg per 100 g, with a mean value of 2.13; the pantothenic acid values ranged from 0.21 to 0.95 mg per 100 g, with a mean value of 0.53.

The mean niacin values for the hybrids, locations, and years are shown in Table II. The corresponding data for pantothenic acid are shown in Table III.

The data shown in Table I were subjected to analysis of variance and the results are shown in Table IV. Highly significant differences ascribable to hybrid, location, and year were found for both vitamins.

TABLE II

MEAN NIACIN VALUES OF CORN HYBRIDS[1] (MILLIGRAMS PER 100 GRAMS)

	Ohio C38	Ill. 201	Ill. 784	Iowa 939	U.S. 13	U.S. 35	U.S. 44	Ind. 608C	Ind. 844D
Wooster, Ohio	2.05	1.90	1.56	2.48	1.88	2.11	2.05	2.25	2.06
Lafayette, Ind.	2.21	2.15	1.76	2.62	1.98	2.28	2.29	2.38	2.38
Urbana, Ill.	2.08	2.09	1.73	2.46	2.11	2.17	2.15	2.31	2.39
Ames, Iowa	2.06	2.11	1.73	2.59	2.04	2.04	2.21	2.35	2.45
Lincoln, Neb.	2.28	1.77	1.65	2.51	1.71	1.93	1.89	2.12	2.08
1943	2.06	1.99	1.80	2.36	2.00	2.18	2.11	2.14	2.21
1944	2.32	1.96	1.66	2.62	2.04	1.97	2.07	2.36	2.28
1945	2.02	2.13	1.59	2.65	1.81	2.21	2.24	2.40	2.38
Three-year means	2.13	2.02	1.69	2.54	1.95	2.11	2.13	2.29	2.29

LOCATION MEANS

	Wooster, Ohio	Lafayette, Ind.	Urbana, Ill.	Ames, Iowa	Lincoln, Neb.	Year mean all locations
1943	2.08	2.09	2.23	2.06	2.00	2.09
1944	1.99	2.23	2.23	2.27	1.97	2.14
1945	2.04	2.35	2.05	2.19	—	2.16
Three-year means	2.04	2.22	2.17	2.17	1.99	2.13

[1] Values are expressed on a 10% moisture basis.

TABLE III

MEAN PANTOTHENIC ACID VALUES FOR CORN HYBRIDS[1] (MILLIGRAMS PER 100 GRAMS)

	Ohio C38	Ill. 201	Ill. 784	Iowa 939	U.S. 13	U.S. 35	U.S. 44	Ind. 608C	Ind. 844D
Wooster, Ohio	0.46	0.49	0.52	0.66	0.40	0.62	0.45	0.44	0.49
Lafayette, Ind.	.55	.55	.48	.68	.45	.52	.42	.46	.40
Urbana, Ill.	.56	.64	.48	.66	.53	.47	.47	.53	.49
Ames, Iowa	.62	.56	.57	.59	.54	.53	.54	.59	.54
Lincoln, Neb.	.52	.56	.52	.59	.54	.53	.51	.53	.67
1943	.59	.66	.49	.67	.60	.57	.52	.48	.53
1944	.46	.36	.37	.47	.35	.46	.39	.47	.49
1945	.56	.69	.72	.80	.53	.58	.52	.59	.49
Three-year means	.54	.56	.51	.64	.49	.53	.48	.51	.51

LOCATION MEANS

	Wooster, Ohio	Lafayette, Ind.	Urbana, Ill.	Ames, Iowa	Lincoln, Neb.	Year mean all locations
1943	0.53	0.50	0.57	0.54	0.70	0.57
1944	.37	.40	.45	.50	.40	.42
1945	.61	.60	.57	.65	—	.61
Three-year means	.50	.50	.53	.57	.55	.53

[1] Values are expressed on a 10% moisture basis.

TABLE IV

VARIANCES FOR NIACIN AND PANTOTHENIC ACID CONTENT OF CORN HYBRIDS

Source of variance	Degrees of freedom	Niacin	Pantothenic acid
Location	4	0.2276**	0.0220**
Hybrid	8	.7907**	.0325**
Year	2	.0435 *	.3950**
Location × hybrid	32	.0217**	.0084
Location × year	8	.0769**	.0341**
Hybrid × year	16	.0851**	.0242**
Error	54	.0089	.0052
Total	124		

* Significant.
** Highly significant.

All three simple interactions indicated highly significant differences in niacin and pantothenic acid, with the exception of the interaction location × hybrid in the latter vitamin.

Tables V, VI, and VII, respectively, show the analysis of variance of the data for separate hybrids, years, and locations.

TABLE V

VARIANCES FOR NIACIN AND PANTOTHENIC ACID CONTENT OF CORN HYBRIDS—INDIVIDUAL HYBRIDS

Source of variance	NIACIN								
	Ohio C38	Illinois 201	Illinois 784	Iowa 939	U.S. 13	U.S. 35	U.S. 44	Indiana 608C	Indiana 844D
Location	0.0259	0.0637*	0.0186	0.0146	0.0600	0.0388*	0.0592*	0.0254	0.0951*
Year	.1127	.0321	.0538	.1197	.0523	.0799**	.0363	.0914**	.0323
Error	.0251	.0136	.0182	.0338	.0446	.0080	.0080	.0085	.0225
	PANTOTHENIC ACID								
Location	0.0101	0.0085	0.0046	0.0049	0.0110	0.0087	0.0067	0.0113	0.0228
Year	.0196*	.1536**	.1407**	.1110*	.0781*	.0207	.0281	.0185*	.0026
Error	.0031	.0151	.0073	.0147	.0151	.0065	.0063	.0033	.0162

* Significant.
** Highly significant.

TABLE VI

VARIANCES FOR NIACIN AND PANTOTHENIC ACID CONTENT OF CORN HYBRIDS GROWN FOR THREE YEARS

Source of variance	Niacin			Pantothenic acid		
	1943	1944	1945	1943	1944	1945
Location	0.0627**	0.1803**	0.1845**	0.0563**	0.0239**	0.0012
Hybrid	.1260**	.4243**	.4106**	.0224**	.0153**	.0431**
Error	.0117	.0268	.0111	.0064	.0039	.0099

* Significant.
** Highly significant.

TABLE VII

VARIANCES FOR NIACIN AND PANTOTHENIC ACID CONTENT OF CORN HYBRIDS GROWN AT FIVE LOCATIONS

Source of variance	NIACIN				
	Ohio	Indiana	Illinois	Iowa	Nebraska
Hybrid	0.1944**	0.1867**	0.1369**	0.1997**	0.1595**
Year	.0192	.1422**	.0937*	.0949 *	.0020
Error	.0128	.0336	.0314	.0250	.0265
	PANTOTHENIC ACID				
Hybrid	0.0211 *	0.0223 *	0.0150	0.0025	0.0049
Year	.1321**	.0851**	.0402	.0572 *	.4252**
Error	.0057	.0084	.0155	.0118	.0062

* Significant.
** Highly significant.

Variations Due to Hybrid. The analysis of variance for the study as a whole (Table IV) showed a greater variation in niacin ascribable to hybrid than to any other factor.

The niacin content of Iowa 939 was the highest (mean 2.54 mg per 100 g) for all three years and at all locations. Illinois 784 assayed the lowest (1.69 mg per 100 g) in niacin for all three years and at all locations (Table II). Highly significant differences were observed within each year (Table VI) and at each location (Table VII). These hybrid differences indicate that the ability of the plant to elaborate niacin is inherited, but may be somewhat modified by environmental factors. However, at one location, Ohio (Table V), the hybrid differences were more highly significant than at any other location; likewise the hybrid differences during 1945 were more highly significant than for either 1943 or 1944.

The greatest variation in pantothenic acid was not ascribable to hybrid (Table IV), although a highly significant difference was due to this factor.

Iowa 939 also assayed highest (0.64 mg per 100 g) in pantothenic acid during each year and at each location. Hybrids assaying low in pantothenic acid were U.S. 13 (0.49 mg) and U.S. 44 (0.48 mg) (Table III).

In the analysis of variance of the data for separate years (Table VI), hybrid caused a highly significant difference in pantothenic acid within each year. Hybrid differences for pantothenic acid were significant in Ohio and Indiana but not in the other states (Table VII).

Variations Due to Year. The variation in niacin due to year (season) was significant for the whole experiment (Table IV). This

factor caused highly significant differences in niacin in U.S. 35 and Indiana 608C (Table V). This factor also caused a highly significant difference in Indiana and a significant difference in Iowa (Table VII).

The greatest variation found in pantothenic acid was ascribable to year (Table IV). The year 1944 produced corn having much less pantothenic acid (0.42 mg per 100 g) than that grown in 1943 (0.57 mg) and 1945 (0.61 mg) (Table III).

The analysis of data for separate hybrids (Table V) showed a highly significant difference in pantothenic acid due to year for Illinois 201 and Illinois 784; significant differences for Ohio C38, Iowa 939, U.S. 13, and Indiana 608C. Year (season) caused highly significant differences in pantothenic acid in Ohio, Indiana, and Nebraska, and a significant difference in Iowa (Table VII). This indicates that the inherited ability of the plant to elaborate pantothenic acid was greatly influenced by environmental factors.

Variations Due to Location. In the analysis of the entire data highly significant differences in both niacin and pantothenic acid were ascribable to location (Table IV), although, as was stated earlier, the greatest variation in niacin was due to hybrid and the greatest variation in pantothenic acid was due to year (season). Location had a greater influence on niacin than on pantothenic acid. Corn grown at the Indiana location was highest in niacin (2.22 mg per 100 g) and that grown at the Nebraska location was lowest (1.99 mg) (Table II). Corn grown at the Iowa location was highest in pantothenic acid (0.57 mg), while that grown at the Indiana and Ohio locations was lowest in pantothenic acid (0.50 mg) (Table III).

In the analysis of the data for separate hybrids (Table V) significant differences in niacin ascribable to location were found only for Illinois 201, U.S. 35, U.S. 44, and Indiana 844D; the differences in pantothenic acid due to location were not significant.

The analysis of the data for separate years (Table VI) showed that location caused highly significant differences in niacin during each of the three years and in pantothenic acid during 1943 and 1944.

Summary

Samples of nine double-cross corn hybrids grown at five experiment stations for two years, and the same hybrids grown for an additional year at four of the five stations, were assayed for niacin and pantothenic acid. Variations for both vitamins were found in all hybrids for all three years and at all locations.

The niacin content of Iowa 939 averaged the highest for all years (2.54 mg per 100 g) and locations, while Illinois 784 assayed the lowest (1.69 mg per 100 g) in niacin.

The niacin values for all samples ranged from 1.43 to 2.95 mg per 100 g, with a mean value of 2.13 mg.

Iowa 939 had the highest pantothenic acid content (mean 0.64 mg per 100 g), and U.S. 44 and U.S. 13 were low in pantothenic acid.

The pantothenic acid values for all samples ranged from 0.21 to 0.95 mg per 100 g, with a mean value of 0.53 mg.

In the analysis of variance for niacin, the greatest variation was ascribable to hybrid.

The greatest variation in pantothenic acid was ascribable to year (season).

The influence of hybrid and year factors on niacin and pantothenic acid, respectively, indicated that the inherited ability of the plant to elaborate niacin was less subject to the influence of environmental factors than was the inherited ability of the plant to elaborate pantothenic acid.

Acknowledgments

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RELATIONSHIP BETWEEN MOISTURE CONTENT OF FLOUR AND HUMIDITY OF AIR

LOREN MOREY, HELEN KILMER,[1] and ROLAND W. SELMAN[2]

(Presented at the annual meeting, May 1947; received for publication May 23, 1947)

Early workers observed that there is a close relationship between the physical properties of flour and the humidity of the atmosphere, and in recent times several authors have reported on the relationship existing between the moisture content of flour and the humidity of the atmosphere surrounding the flour at controlled temperatures. Bailey (1920) and Anker, Geddes, and Bailey (1942) have presented a comprehensive review of the moisture content of wheat flour at various relative humidities. Fairbrother (1929) investigated the weight of flour as related to relative humidity.

No information on the relationship between the moisture content of flour and the atmospheric humidity at temperatures appreciably higher than room temperature has been found in the literature. The object of the present investigation was to supply information on the flour moisture content-humidity-temperature relationship over an extended range. A simple method has been employed to determine the variation of flour moisture content over the range of 50° to 95°C at humidities ranging from approximately zero to 80% relative humidity.

Materials and Methods

A typical southwestern wheat flour analyzing 11.5% protein and 0.45% ash (14% moisture basis) was used in this study. An amylograph test made according to the procedure of Selman and Sumner (1947) gave a malt index of 500; hence the flour was adequately diastated.

TABLE I

RELATIVE HUMIDITY OF AIR IN EQUILIBRIUM WITH SULFURIC ACID SOLUTIONS AT 50°, 75°, AND 95°C

Solution	Percentage H_2SO_4 (by weight)	Relative humidity of equilibrium air		
		50°C	*75°C*	*95°C*
A	26.12	80.1	81.6	82.1
B	40.08	58.1	59.1	59.8
C	52.27	33.1	35.0	37.3
D	83.58	0.44	0.68	0.97

For each temperature and relative humidity 2 g of flour were weighed in quadruplicate into a glass-stoppered weighing bottle, and,

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with the stopper removed, placed in a laboratory desiccator containing 170 ml of a sulfuric acid solution which would give the desired relative humidity. The desiccator was then stored in a laboratory oven at the desired temperature, and the weight of each sample was checked periodically until the weight became constant.

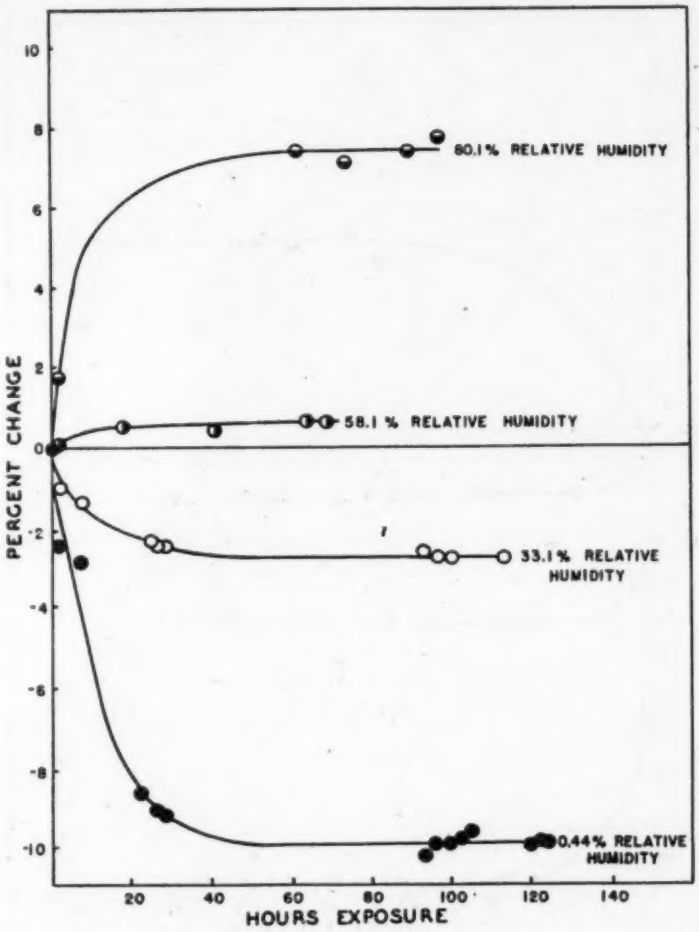

Fig. 1. Moisture variation of flour at 11.36% moisture content upon exposure at 50°C to air of different relative humidities.

The sulfuric acid solutions used were made from reagent grade concentrated sulfuric acid, the concentrations of which were determined by means of both hydrometers and titration against standard sodium carbonate solutions. The relative humidity of air in equilibrium with each of the solutions was calculated from the data of Wilson (1921)

and from the International Critical Tables (1928). The calculations are recorded in Table I.

At the completion of the experiment the specific gravity of the sulfuric acid solution was checked. In every experiment, there was less than 1% change in the specific gravity.

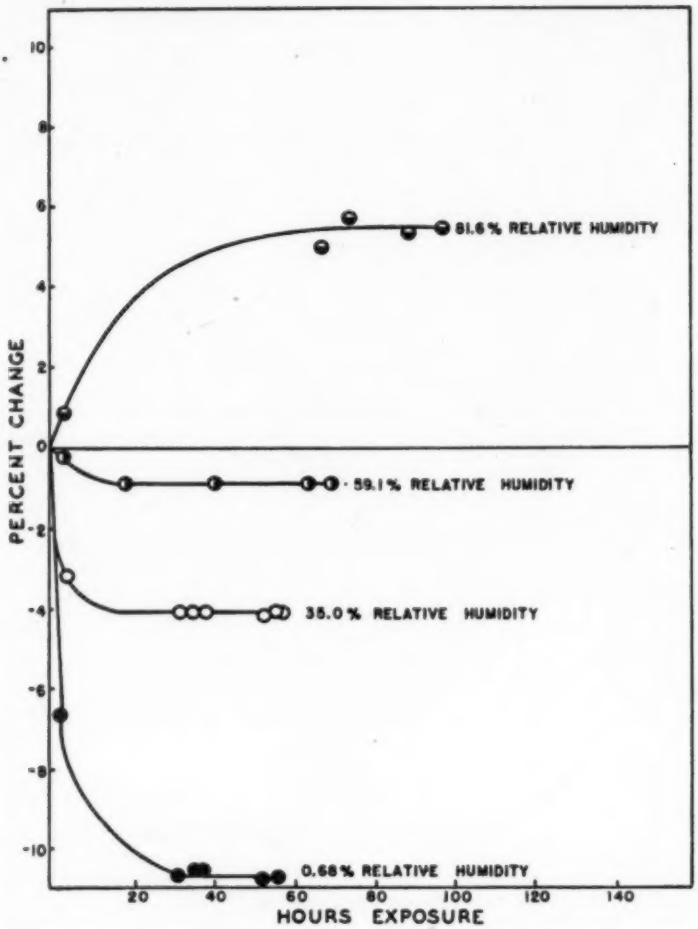

Fig. 2. Moisture variation of flour at 11.36% moisture content on exposure to air at 75°C.

Results and Discussion

The percentage change in the moisture content of the flour was plotted as related to time in the oven. Figures 1, 2, and 3 show the results. The values are based on the average of quadruplicate determinations.

The original moisture content of the flour was determined by the air-oven method and was found to average 11.36%. The data of Figure 1 show that about 50 hours are required for all samples to reach equilibrium at 50°C and 75°C as compared with about 40 hours of exposure for the flour at 95°C. However, as the temperature increased

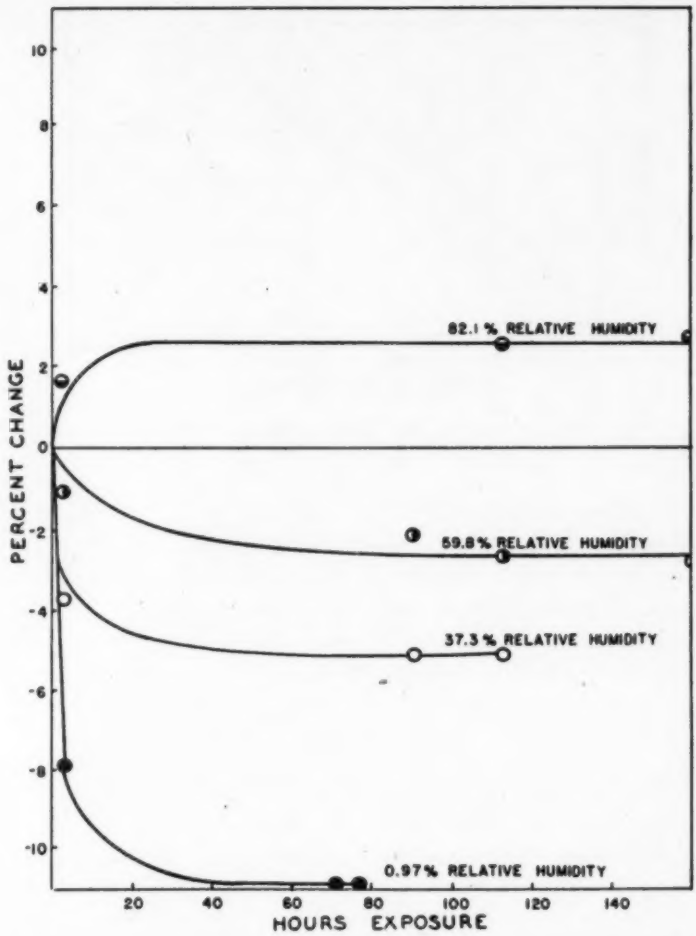

Fig. 3. Moisture variation of flour at 11.36% moisture content on exposure to air at 95°C.

the time required for reaching equilibrium at any given humidity was diminished somewhat.

The relationship existing between the relative humidity of air and the moisture content of the flour is represented graphically in Figure 4 and numerical data for selected relative humidities are shown in Table II.

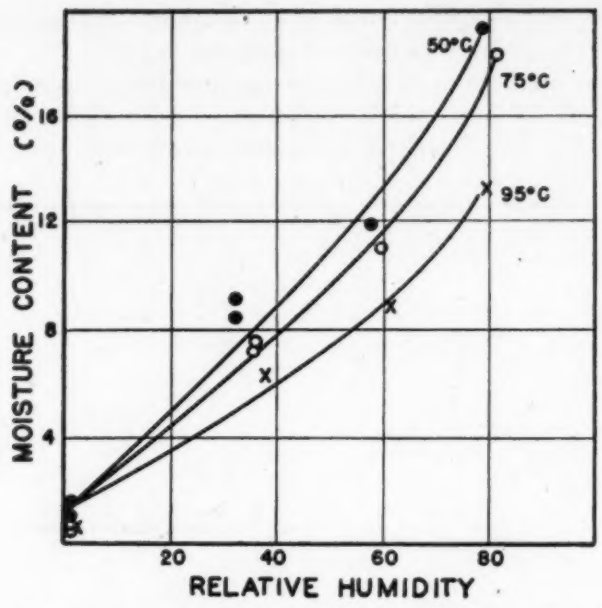

Fig. 4. Moisture content of flour at 50°C, 75°C, and 95°C in equilibrium with atmospheres of different relative humidities.

The moisture content of flour in equilibrium with air of low relative humidity varies little with the temperature. With increasing relative humidities the moisture contents increased at a rate slightly more than linearity at all temperatures within the range studied.

Although it is customary to express the moisture content of air in terms of the degree of saturation of the air, i.e., relative humidity, a

TABLE II

MOISTURE CONTENT OF FLOUR IN EQUILIBRIUM WITH AIR OF VARIOUS RELATIVE HUMIDITIES AT DIFFERENT TEMPERATURES

Air temperature = 50°C			Air temperature = 75°C			Air temperature = 95°C		
Air moisture		Flour moisture	Air moisture		Flour moisture	Air moisture		Flour moisture
R. H.	Water vapor pressure		R. H.	Water vapor pressure		R. H.	Water vapor pressure	
%	*mm Hg.*	%	%	*mm Hg.*	%	%	*mm Hg.*	%
80.1	75.5	18.75	81.6	236	16.58	82.1	520	13.96
58.1	53.7	10.98	59.1	171	10.57	59.8	379	8.63
33.1	30.6	8.19	35.0	100	6.85	37.3	237	6.24
		8.56			7.20			
0.44	4.06	0.91	0.68	19.6	0.36	0.97	61.5	0.48
		1.41			0.64			

more absolute definition of the value is the water vapor pressure in millimeters of mercury. Figure 5 demonstrates that over the range studied the moisture content of the flour is a linear function of the water vapor pressure for a given temperature.

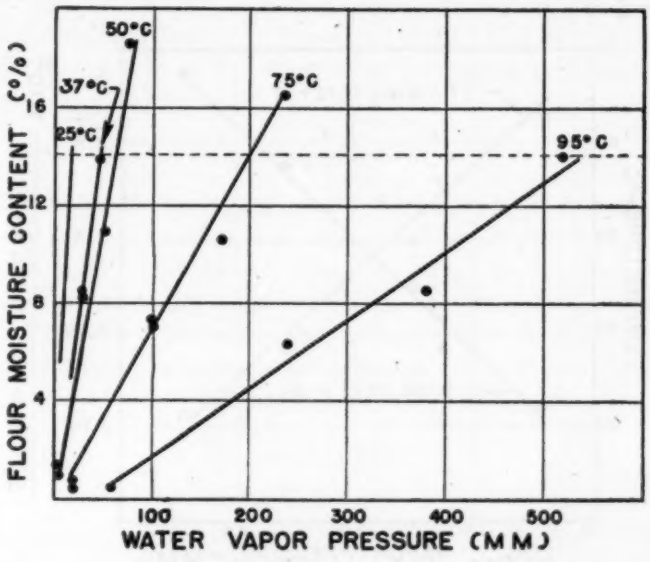

Fig. 5. Isothermal relationships of flour moisture content to water vapor pressure.

In addition to the experimental results presented in this paper the moisture content of flour in equilibrium with air of various humidities at 25° and 37°C has been calculated from the data of Anker, Geddes, and Bailey (1942), and is included in Figure 5. The isothermal relationships between the flour moisture content and the water vapor pressure have been computed by the method of least squares and the regression coefficients and ordinate intercepts are listed below.

Temperature, degrees C	*Regression coefficient*	*Ordinate intercept*
25	0.585	4.08
37	0.302	0.249
50	0.233	0.433
75	0.0720	−0.0721
90	0.0283	−1.13

Since commercial flour generally has a moisture content in the neighborhood of 14%, it is interesting to note the water vapor pressure giving this value at all of the temperatures listed in the above table. As shown in Figure 6, the relation between temperature and the logarithm of the water vapor pressure is essentially linear, the regression

equation being:

$$t_{14\%} = 48.6 \log_{10} (\text{V.P.})_{14\%} - 37.4$$

when $t_{14\%}$ and V.P.$_{14\%}$ are the temperature and water vapor pressure, respectively, required to give flour of 14% moisture content.

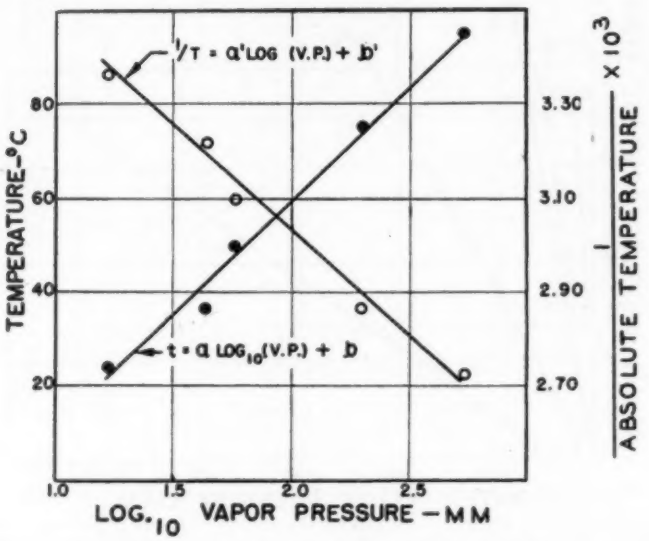

Fig. 6. Relationship of temperature to the water vapor pressure of flour at 14% moisture content.

This evaluation of the data could be extended to any moisture content by interpolation of the graphs to any desired value and relating the temperature to water vapor pressure.

Although practical requirements make it more desirable to express temperature in the units of the Centigrade scale, theoretical considerations reveal that the reciprocal of the absolute temperature is preferable. From the Clausius-Clapeyron equation, the logarithm of the vapor pressure of a liquid is approximately a linear function of the reciprocal of the absolute temperature and the slope of the line expressing this relation can be used to calculate the heat of vaporization. This convenient relationship has been used by Neale and Stringfellow (1941) to determine the heat of sorption of cotton from data similar to those presented in this paper.

Reconsideration of the values shown in Table II show that for the flour at 14% moisture content the relationship is:

$$\frac{I}{T_{14\%}} = 0.00392 - 0.000443 \log_{10} (\text{V.P.})_{14\%}$$

when T is the absolute temperature and V.P. is the water vapor pressure. The equation is graphically represented in Figure 6. From the regression coefficient the heat of dehydration of the flour is calculated as 10.7 Calories per mole of water. Winkler and Geddes (1931) arrived at a value of 2.96 Calories per mole of water as the energy of adsorption at a flour moisture content of 14% in the presence of an excess of liquid water. The value of 10.7 Calories per mole is in reasonable agreement if the heat of vaporization is taken into consideration.

Summary

The equilibrium moisture contents of a typical Kansas flour in contact with air of varying moisture content at 50°, 75°, and 95°C were determined. A linear relationship exists between flour moisture content and the absolute humidity of the air. For a moisture content of 14%, the reciprocal of the absolute temperature is a linear function of the logarithm of the water vapor pressure. The heat of dehydration has been calculated as 10.7 Calories per mole of water.

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IMPROVED FLOUR BLEACHING PROCESS USING CHLORINE DIOXIDE PRODUCED BY AN ELECTRIC ARC METHOD [1]

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(Presented at the Annual Meeting, May 1947; received for publication June 24, 1947)

Nitrogen oxides have been used for the bleaching of flour for many years. Several methods of obtaining these nitrogen oxides at low partial pressures have been proposed and tried commercially; but the passage of air through a continuous electric arc seems to have proved the most successful for flour bleaching purposes. Alsop machines in this country and Brabender equipment in Europe employing the electric arc method have been in wide use for a long time. Many of these generators are in use at the present time; but others are idle in mills or warehouses. The reasons for the decreased usage of the process in this country in the past decade or so are twofold. First, as shown by Ferrari, Hutchinson, and Mecham (1945) the color removal with nitrogen oxides is not nearly as great as that which can be achieved with nitrogen trichloride and benzoyl peroxide. Second, the desirable effects on the baking properties of certain types of flours that are achieved with nitrogen trichloride and chlorine dioxide cannot be brought about by the application of nitrogen oxides from the electric arc machines to flours.

The purpose of this paper is to show how the electric arc generators for producing nitrogen oxides can be converted to bleaching generators capable of producing chlorine dioxide or a mixture of chlorine dioxide and nitrogen oxides diluted with air. A greatly improved bleaching process results. Flexibility in the proportions of the two reagents, a much greater degree of color removal, and desirable maturing effects on the flour resulting in improved baking properties are achieved, completely overcoming the above noted drawbacks to the nitrogen oxide bleaching process. The effects of chlorine dioxide on the baking properties of flour have been discussed by Ferrari, Hutchinson, Croze, and Mecham (1941). In most instances they are quite similar to those produced by nitrogen trichloride.

Equipment and Procedure

Much of the equipment and many of the procedures used in these experiments were described in the recent paper by Hutchinson, Derby,

[1] Paper No. 81, Journal Series, General Mills, Inc., Research Laboratories.

and Ferrari (1947). A diagram of the equipment for the small-scale experiments is shown in Figure 1.

This process for producing chlorine dioxide is controlled by generating known amounts of nitrogen oxides (Hutchinson *et al.*, 1947) and contacting them with a large excess of technical grade flaked sodium chlorite (such as Mathieson Alkali Works, Inc., "C_2") in a vertical column. For the small-scale experiments reported in this paper the effluent gases from the Alsop equipment were proportioned by flowmeters, the needed amount being conducted to the chlorite tower and the excess being exhausted to a hood or the outside atmosphere. In

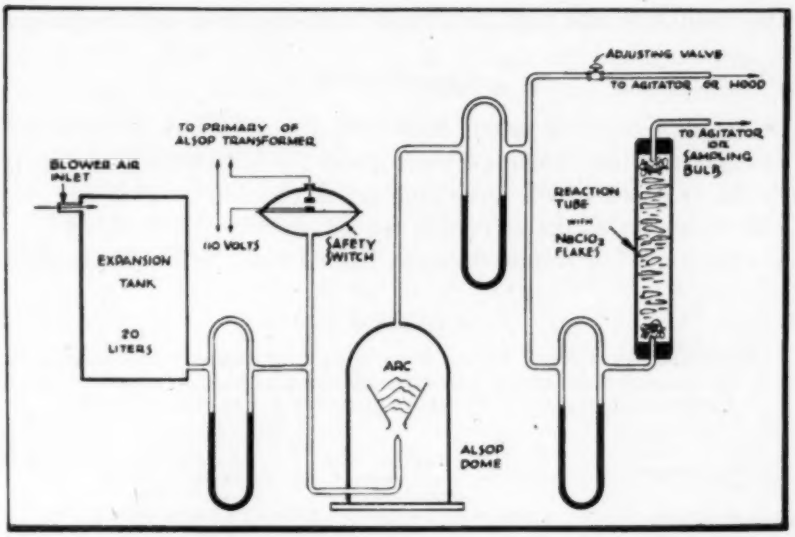

Fig. 1. Diagram of the pilot scale equipment used in the manufacture of ClO_2 gas with the Alsop generator.

commercial application there would be no need for wasting any of the Alsop gases. Continuous operation would be achieved by the use of a multiplicity of chlorite towers, the nitrogen oxides being introduced to some of them, while the others were being charged with fresh chlorite. Suitable multiple chlorite tower equipment has been described by Woodward, Petroe, and Vincent (1944).

The effluent gases from the chlorite towers, consisting of chlorine dioxide or a mixture of chlorine dioxide and nitrogen oxides, were conducted to MacLellan Batch Mixers for the flour bleaching process.

One of the outstanding advantages of the process is the apparent ease of safeguarding the generation of chlorine dioxide in this manner. The Alsop equipment is only capable of generating nitrogen oxides at low partial pressures of 3 mm Hg or less when operating at optimum

capacities. Moreover, the current to the electrodes forming the arc is shut off by means of a diaphragm contact activated by the air supply to the nozzle, when the air pressure to the nozzle beneath the arc falls below a predetermined level. Although the exact course of the reaction between the nitrogen oxides and the chlorite has not been determined, it appears that about one mole of chlorine dioxide is produced from each mole of nitrogen peroxide. Thus, the partial pressure of the chlorine dioxide is automatically kept at about 3 mm Hg or less, far below the safe partial pressure for chlorine dioxide which is about 30 mm Hg according to Woodward *et al.* (1944). The necessary equipment for safeguarding the process is therefore already available in the Alsop machine with the air pressure-operated contact switch.

Experimental

Some samples of a patent flour milled from a spring wheat blend were experimentally bleached with Alsop gases. Others were treated with Alsop gases which were conducted through a 40-inch column 1 5/16 inches in diameter containing Mathieson Alkali Works, Inc., "C_2" chlorite. The results shown in Table I indicate that the bleaching

TABLE I

BLEACHING OF PATENT FLOUR WITH NITROGEN PEROXIDE PRODUCED IN AN ALSOP MACHINE AND WITH ALSOP GASES CONVERTED TO CHLORINE DIOXIDE BY PASSAGE THROUGH A CHLORITE COLUMN

Bleaching treatment per cwt. of flour	Pekar or "slick" score	"Carotene" [1] p.p.m.	Bread crumb color
Unbleached flour	creamy	1.98	—
0.9 g nitrogen peroxide[2]	7 creamy	1.01	10 creamy
0.9 g nitrogen peroxide-repeat	7 creamy	1.05	—
0.9 g nitrogen peroxide through a column of chlorite[3]	10 white	0.77	12 white
Repeat of above treatment	10 white	0.77	—
1.0 g nitrogen peroxide through the chlorite column	10 white	0.69	—

[1] Flour color expressed as naphtha-alcohol "carotene"; determined as outlined in *Cereal Laboratory Methods* (4th ed. 1941).
[2] This sample was used as standard for the bread crumb color tests.
[3] This sample was used as standard for the Pekar tests.

potency of the gases is very greatly increased by passage through the chlorite column. Both the slick and bread crumb colors were improved and whitened. The carotene contents of the treated flours clearly reflected the greater bleaching power due to the conversion of the nitrogen oxides to chlorine dioxide by the passage through the chlorite column. Carotene contents of 0.77 and 0.69 p.p.m. are in the range of commercially bleached flours.

In these preliminary experiments the conversion of the nitrogen oxides in the Alsop gases to chlorine dioxide was not a hundred percent complete; but it was believed to have been substantially so by the bleaching results and by the analyses of effluent gases from the Alsop equipment and from the chlorite tower. The odor of the effluent gases from the chlorite tower indicated the presence of some unreacted nitrogen oxides.

Woodward *et al.* (1944) described a method of analysis for chlorine dioxide gas which involves absorbing the gas in a 10% potassium iodide solution made from chlorine-free water, adding a starch indicator, and titrating to a colorless end point with 0.1 *N* sodium thiosulfate solution. The solution is then acidified with an excess of 30% acetic acid and is again titrated with the same strength sodium thiosulfate. One-fifth of the total sodium thiosulfate corresponding to the chlorine dioxide is used in the first titration and four-fifths is used in the second titration. Thus the ratio of the thiosulfate consumed in the first titration to that in the second is 1:4 for pure chlorine dioxide. Chlorine titrates in the first titration, so the ratio is rapidly increased, if appreciable quantities of chlorine are present.

Analysis of the effluent gases from an Alsop machine, consisting of nitrogen oxides and air predominantly, resulted in a ratio of approximately 1:10, far different from the 1:4 ratio secured with pure chlorine dioxide. Gases obtained by passing the effluent gases from an Alsop machine through a column of sodium chlorite were analyzed in the same manner and the ratio of the first to the second titration value was found to be approximately 1:4. This indicates that the nitrogen oxides were converted practically quantitatively to chlorine dioxide.

The conversion of the nitrogen oxides to chlorine dioxide in this process can probably be expressed by the following equation, although the exact course of the reaction is not known:

$$NO_2 + NaClO_2 \rightarrow NaNO_2 + ClO_2$$

Optimum methods of utilizing chlorine dioxide and nitrogen peroxide in multiple flour bleaching combinations have been described by Hutchinson and Derby (1945). Their data indicate that maximum color removal is achieved by the sequential application of chlorine dioxide followed by nitrogen peroxide. Application in the reverse order or simultaneously results in appreciably less color removal.

Flexibility in bleaching treatments can be achieved by splitting the effluent gases from an Alsop or Brabender machine, conducting one portion through a chlorite column and then to the first flour agitator, and conducting the second portion with no conversion treatment directly to the second flour agitator.

Summary

A process is described for converting the effluent nitrogen oxide gases from electric arc equipment such as the Alsop or Brabender machines to chlorine dioxide by passage through a bed or column of technical grade sodium chlorite. The converted gases exhibit much greater bleaching power and also possess the ability to alter the baking properties of certain types of flours which is characteristic of chlorine dioxide and which is similar in many instances to that possessed by nitrogen trichloride.

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PROTEIN AS A FACTOR IN SOFT WHEAT MEAL MIXOGRAM AREA

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Ohio Agricultural Experiment Station, Wooster, Ohio

(Presented at the Annual Meeting, May 1947; received for publication May 21, 1947)

The mixogram offers possibilities as a means of evaluating and classifying new strains of wheat. In the early stages of a breeding program the number of lines is large and the grain from each very limited in amount. Because of the large number of lines, the time factor makes micro milling unfeasible, but a sifted meal may be used to make mixograms very similar to those made from flour. A method has been described by Lamb (1944) for preparing the meal from small amounts of wheat. Morris, Bode, and Heizer (1944) found that the area under the flour mixogram, measured with a planimeter, has a general relationship to quality of soft wheats and to the type of products for which they may be utilized. Sifted meal mixograms are equally useful for this purpose.

Given mixograms from two strains of wheat, the factor most likely to interfere with comparison of mixogram areas is protein content. Differences in protein, as Swanson (1940) and also other workers have pointed out, affect the height of curve, but have little or no influence on the time required to reach minimum mobility. Consequently the angles of the ascending and descending slopes are steeper in curves from higher protein samples. The area under the mixogram also increases. Measurements of mixogram area then are affected by protein level, and this complicates comparisons. The purpose of the present study was to evaluate this effect of protein on sifted wheat meal mixograms.

Experimental

A series of 21 nursery lines was taken from the advanced nursery tests grown at four stations in Ohio for two years. These selections had been advanced from preliminary tests, and, so far as could be judged from appearance, were of satisfactory soft wheat quality. Mixograms and protein analyses were made for each of the 168 samples. Mixogram areas were measured for a six-minute interval.

Preliminary analysis of the data showed there were highly significant differences in mixogram area. Covariance analysis was then made. When the data were considered as eight station-year groups, thus eliminating variety as a factor, protein differences accounted for nearly all the variations in mixogram area. Figure 1 shows graphically that the protein content did account for differences in mixogram area. The range in meal protein was 9.9 to 12.3%, a range not greater than that encountered in commercial samples of Ohio wheat. The close arraignment to the regression line is not due to an artificially expanded series. Neither station nor season introduced significant variation in mixogram area. Harris, Sibbitt, and Elledge (1944) report analogous data from six spring wheats grown at four stations for two seasons. They found significant differences in curve height between seasons. This does not contradict the present findings, since curve height is closely associated with protein level.

Next, the data were grouped by variety. The covariance analysis showed that protein did not explain all differences in mixogram area between varieties; the area differences, independent of protein, were considerable, and were highly significant statistically. The data are presented graphically in Figure 2.

The data present some further points of interest. In Figure 2, the regression line for mixogram area on protein is indicated for each of the 21 varieties. There is variability in the slopes of these lines, which is not surprising since each regression is determined from only eight samples. Statistically there is no indication that the slopes of the

regression lines are not homogeneous. The average regression therefore should provide a satisfactory measure of the change in mixogram area to be anticipated with different protein levels. This average regression is shown in Figure 2, and indicates an increase of about 4.5 sq cm in mixogram area for each 1% increase in protein of the wheat meal. Adjusting mixogram areas to protein content reduced the mean square for error within varieties from 24.08 to 8.38, and furthermore the reduction in sum of squares due to regression was very large

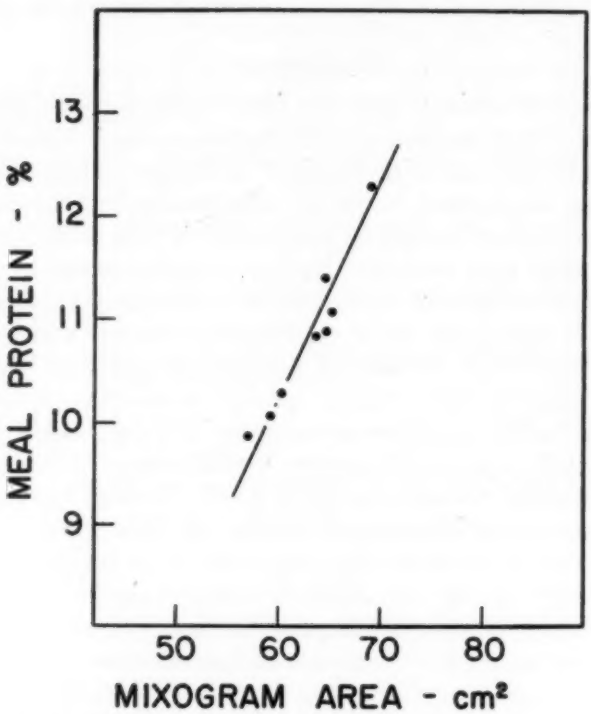

Fig. 1. Relation between meal protein and mixogram area in 8 tests, each including the same 21 varieties of soft red winter wheat.

and highly significant. In Figure 1, the regression from station means agrees very closely with this figure, indicating about 4.6 sq cm for each 1% protein in the wheat meal.

Morris in his work at the Federal Soft Wheat Laboratory (unpublished data) has found a greater increase in soft wheat flour mixogram area for each percent increase in protein, namely 7 sq cm. Most, but not all, of this difference would have been anticipated because Morris measures area under flour mixograms for a seven-minute time interval rather than the six minutes used for sifted meal mixograms,

and flour curves have greater areas even when the same time interval is used. A slightly higher absorption was used for the wheat meal mixograms, and this would also tend to give a smaller increase in area for each increment in protein.

Mixogram area does not give a complete picture of soft wheat quality; it does not even give all the information to be obtained from the mixogram. However, it is considered one of the best single tests

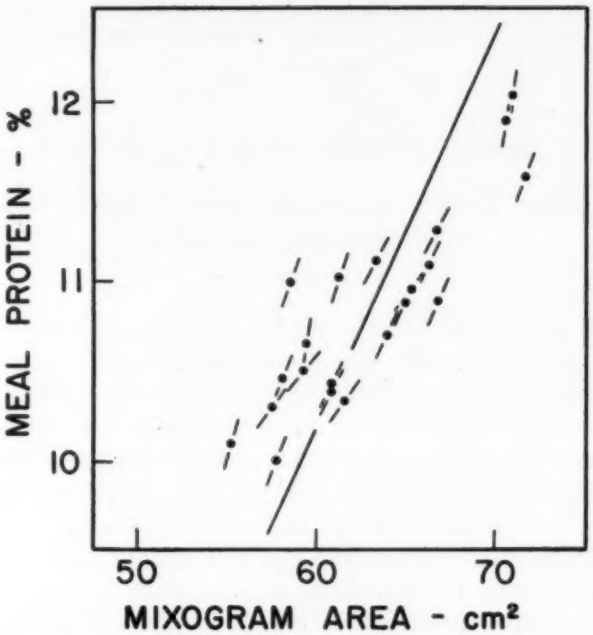

Fig. 2. Relation between meal protein and mixogram area for 21 varieties of soft red winter wheat, each grown in the same 8 tests. Regressions for each variety and average regression are also shown.

that have been developed for evaluating new selections at an early stage in the soft wheat breeding program at the Ohio Station.

Summary

Area under the mixogram is influenced by both variety and protein content. In the case of soft wheats the influence of protein may be reduced to insignificant proportions by adjusting six-minute sifted meal mixogram areas 4.5 sq cm for each 1% protein. When the influence of protein has been largely eliminated in this way there remain highly significant differences in area which are due to variety, and which may be used as a measure of quality differences.

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COMMUNICATION TO THE EDITOR

A Spot Test for the Identification of Rodent Urine on Packaged Commodities

Received June 8, 1947

SIR:

The use of the "black light" for the detection of rodent contamination on bagged commodities has presented some difficult problems in that many ingredients used in the milling and baking industry fluoresce under ultraviolet light. While each substance has its characteristic color, many are of a similar shade, often confusing the operator. Three of the common tests for urine are the urease test for urea, the xanthydrol test for urea, and the extraction of urea and crystallization of urea nitrate (*Methods of Analysis A.O.A.C.*). These tests all involve cutting out a portion of the stained cloth, chemical treatment, and observation of characteristic crystals under the microscope.

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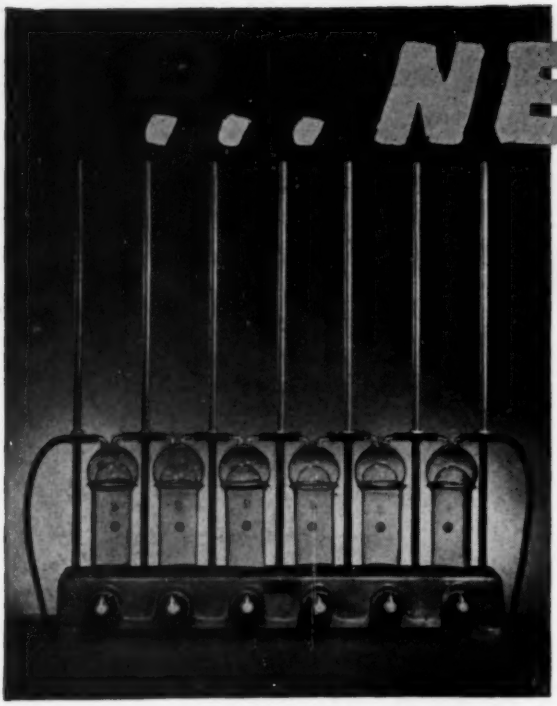

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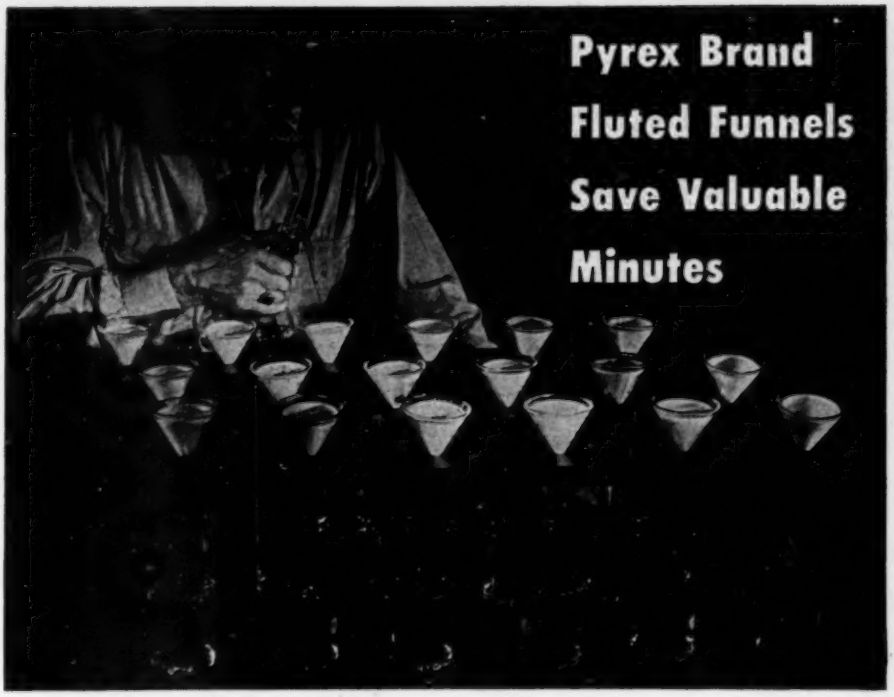

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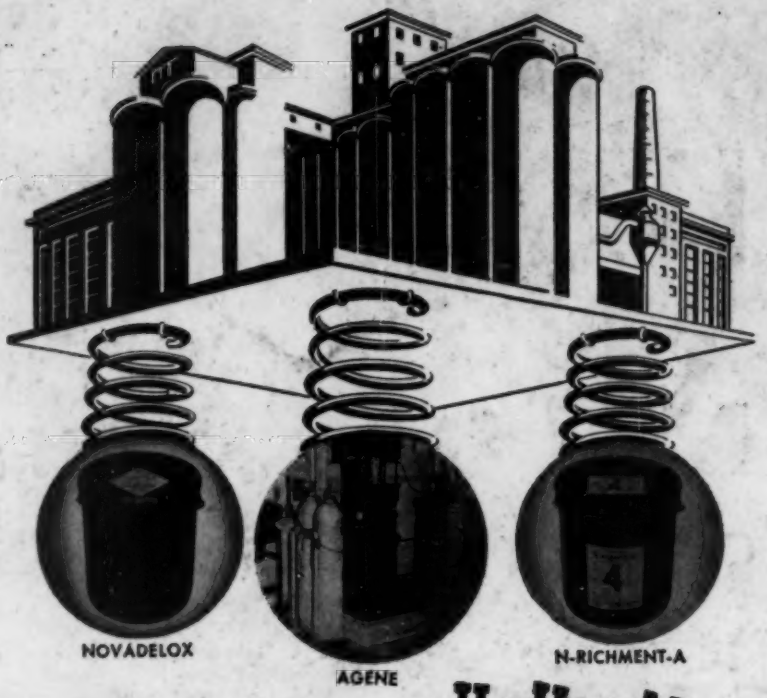

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